Analyst® 1.6 Software

Scripts User Guide

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ABSCIEX

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Foreword

This guide provides information on how to use the Analyst® software scripts and is intended for customers and FSEs.

Related Documentation

The guides and tutorials for the instrument and the Analyst® software are installed automatically with the software and are available from the Start menu: All Programs > AB SCIEX > Analyst. A complete list of the available documentation can be found in the online Help. To view the Analyst software Help, press F1.

Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the Web site at www.absciex.com.

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The purpose of this document is to explain how to install and use Analyst® software scripts. It also provides an overview of the uses of each script and how to uninstall a script, if required.

Related Documentation

You can also create your own scripts using the Visual Basic Version 6 program. For more information, see the *Analyst® Software Automation Cookbook: A guide to controlling Analyst® software version 1.6 from Visual Basic .NET Version Visual Studio-2005*.

Installing or Uninstalling Scripts

Some scripts are automatically installed when the Analyst software is installed. The remaining scripts are available in the Scripts folder. When you have decided which scripts you want to use, use the following procedure to install them.

Note: This guide contains the scripts for all instruments and different software versions. To determine which scripts the software version installed on your instrument supports, see the Analyst software release notes.

Installing a Script

1. Navigate to the following folder on your workstation: <drive>:\Program Files\Analyst\Scripts.
2. Open the required folder, open the script folder, and then double-click **ScriptRunner.exe**.
3. Follow the on-screen instructions to install the scripts.

   The script is available on the Script menu.

Note: For some scripts, if you hold down the Shift key while accessing a script on the Script menu, a description of the script is displayed.

Uninstalling a Script

- To uninstall a script, do one of the following:
  - For processing scripts, navigate to the <drive>:\Analyst Data\Projects\API Instrument\Processing Scripts folder and then delete the script .dll or, if applicable, .exe and .bmp files, manually.
  - For acquisition scripts, navigate to the <drive>:\Analyst Data\Projects\API Instrument\Acquisition Scripts folder and then delete the script .dll or, if applicable, .exe and .bmp files, manually.

Add Missing Zeros

Use this script to add a value of zero intensity for the missing mass values in the spectrum. To minimize storage requirements and to speed data display and processing, the Analyst® software does not store or display spectral points with an original intensity of zero. If required, for example, when exporting a spectrum for subsequent processing by custom software, you can add these data points back to the spectrum.

To use the script

- Click the spectrum and then click **Script** > **Add Missing Zeros**.

  The script will add the zero values to all missing masses in the current spectrum.

Add Normalized ADC Traces

Use this script to overlay the active chromatogram or chromatograms with normalized ADC data from a corresponding data file. Run the script when a pane containing one or more chromatograms is active in Explore mode. There may be a time region selected in a trace. If no selection is made, then the entire chromatogram is overlayed.

In case that several ADC traces are part of the .wiff file, all of them will be displayed.

Notes

- If the data in the active pane came from several samples, then the ADC data for the sample corresponding to the first data set (not the active data set) is shown.

To use the script

- Do one of the following:
  - Click **Script** > **AddNormalizedADC**.
  - To see the script description and add ADC data to an active Explore pane, hold the Shift key down while clicking the script.

Analyst 1.2 Peak Finder Parameters

The Analyst® software uses an improved version of Peak Finder for better peak detection and ion abundance measurement. If you are using the Peak Finder algorithm to analyze .wiff files that were acquired using the Analyst software, then you can use this script to set the peak finder algorithm parameters.

To install the script

- Make sure that the Analyst Data\Projects\API Instrument\Processing Scripts folder contains the Analyst12PeakFinderParams.dll.

Note: This script is installed automatically when the Analyst 1.6 software is installed. There is no separate installation program for it.

To use the script

1. Click **Script** > **Analyst12PeakFinderParams**.

   The Analyst 1.2 Peak Finder Algorithm Parameter dialog appears.

2. To activate the Analyst 1.2 version of Peak Finder, select the **Use Analyst 1.2 peak finder algorithm for Analyst 1.2 files** check box.

3. Do the following:

   - In the **Intensity Threshold (%)** field, type the value, as a percentage, of the minimum intensity required to distinguish between noise and peak.
   - In the **Centroid Height (%)** field, type the value, as a percentage, to be used by the centroiding algorithm to find the peak and to determine the centroid *m/z* value at this percentage height.
   - In the **Centroid Peak Width (min)** field, type the minimum value, in ppm, to be used by the centroiding algorithm to find the peak width and to determine the centroid *m/z* value at this width.
   - In the **Centroid Peak Width (max)** field, type the maximum value, in ppm, to be used by the centroiding algorithm to find the peak width and to determine the centroid *m/z* value at this width.
   - In the **Centroid Merge Distance (amu)** field, type a value, in amu, to be used to determine whether two centroid peaks should be merged into one. If two peaks are within this tolerance, then they will be merged together.
   - In the **Centroid Merge Distance (ppm)** field, type a value, in ppm, to be used to determine whether two centroid peaks should be merged into one. If two peaks are within this tolerance, then they will be merged together.

4. Click **OK**.

5. To return to the Analyst 1.4.1 Peak Finder algorithm, clear the **Use Analyst 1.2 peak finder algorithm for Analyst 1.2 files** check box.

Batch Script Driver

With the Batch Acquisition Script Driver, you can run an acquisition script on multiple data files (.wiff) by attaching the acquisition script to a batch that is submitted to the queue. These scripts are used to immediately process the data either after a sample completes or after the batch finishes. The script will process the data files as if they were separate batches submitted to the queue. Occasionally, you might need to apply these acquisition scripts on previously acquired data because either the script did not exist at the time of acquisition or a rerun of the script is required.

To use the script

1. Navigate to C:\Program Files\Analyst\bin and then double-click **BatchScriptDriver.exe**.

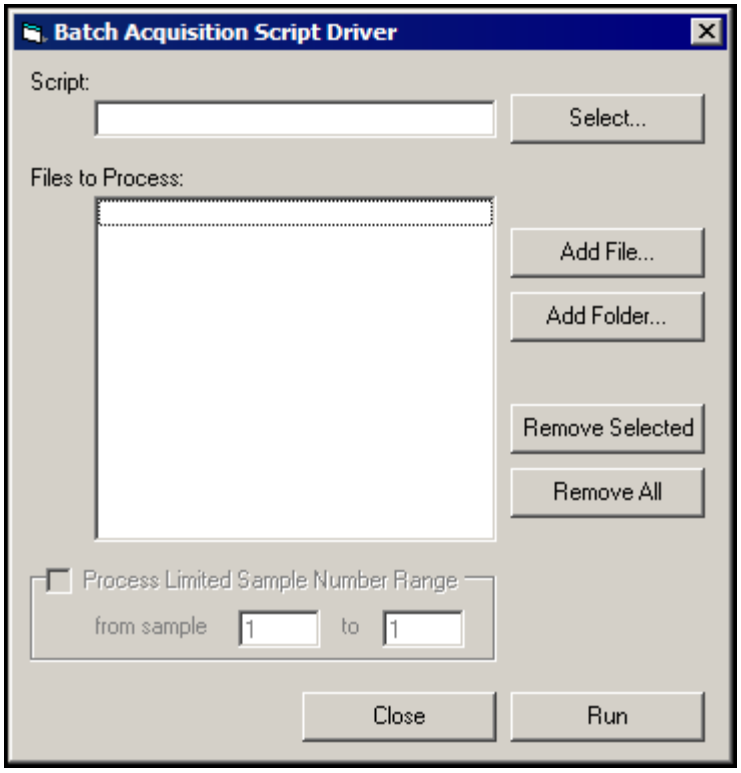


Figure 1-1 Batch Acquisition Script Driver dialog

2. To select an acquisition script, click **Select**.
3. To add a data file, click **Add File**. Click **Add Folder** to add an entire folder of data files.
4. To remove a data file, select it from the list and then click **Remove Selected**. Click **Remove All** to remove all the data files.
5. To process some of the samples contained in a .wiff data file, select the **Process Limited Sample Number Range** check box. This field is available only if you have selected only one data file to process.
6. In the **from sample** and **to** fields, type the sample number range to be processed.

7. To run the script on each data file in the list, click **Run**.
8. To stop the script, click **Close**.

Change All Methods

It is often necessary to change the ion source conditions of a method, particularly with the NanoSpray® ion source after changing the emitter tip. This script modifies every method in a selected project with new values for IonSpray Voltage (IS), Ion Source Gas 1 (GS1), and Interface Heater Temperature (IHT).

Note: This script is used with any QTRAP® system. It is not used by QSTAR® instruments.

To use the script

1. Click **Script** > **change all methods**.

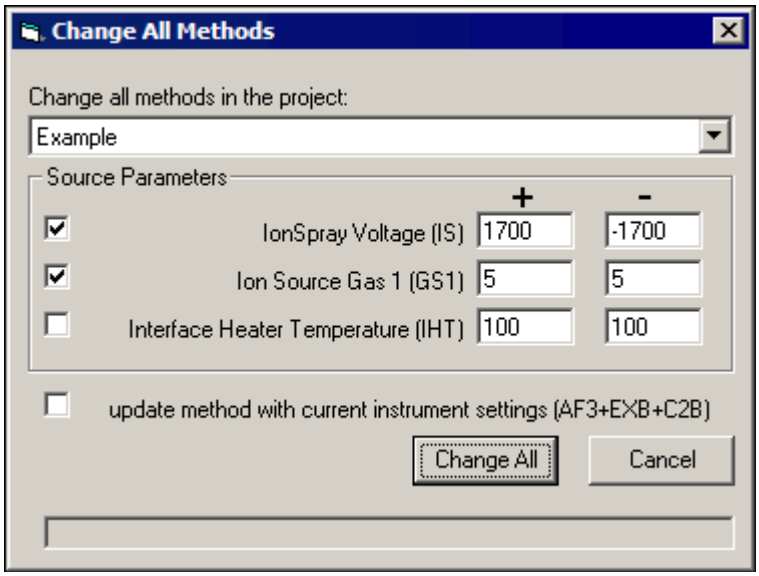


Figure 1-2 Change All Methods dialog

2. Select a project containing the methods that you want to modify.
3. Select the parameters that you want to change. If the parameter is not in the method file, it will be ignored.
4. Type a value for positive experiments.
5. Type a value for negative experiments.
6. Select the **update method with current instrument settings** check box if you want to change the **AF3**, **EXB**, and **C2B** parameters for all the methods in the selected project.
7. Click **Change All**.

Convert Methods

Use this script to convert methods from one type of instrument to another. The script converts the method to the currently active hardware profile, using appropriate values for each parameter. Only the ion source and compound dependent parameters for mass ranges and experiments are shown.

The Convert Methods script automatically optimizes mass ranges and, in addition to single period, single experiment methods, converts multiple periods, multiple experiments, and IDA criteria.

Prerequisites

- .Net framework 3.5 SP2 (will be automatically installed if required)

To install the script

- To install the script, navigate to the <drive>:\Program Files\Analyst\Scripts\Convert Methods folder and then double-click **Convert Methods Setup.exe**.

To use the script

Make sure a hardware profile is active.

1. Click **Script** > **Convert Methods**.

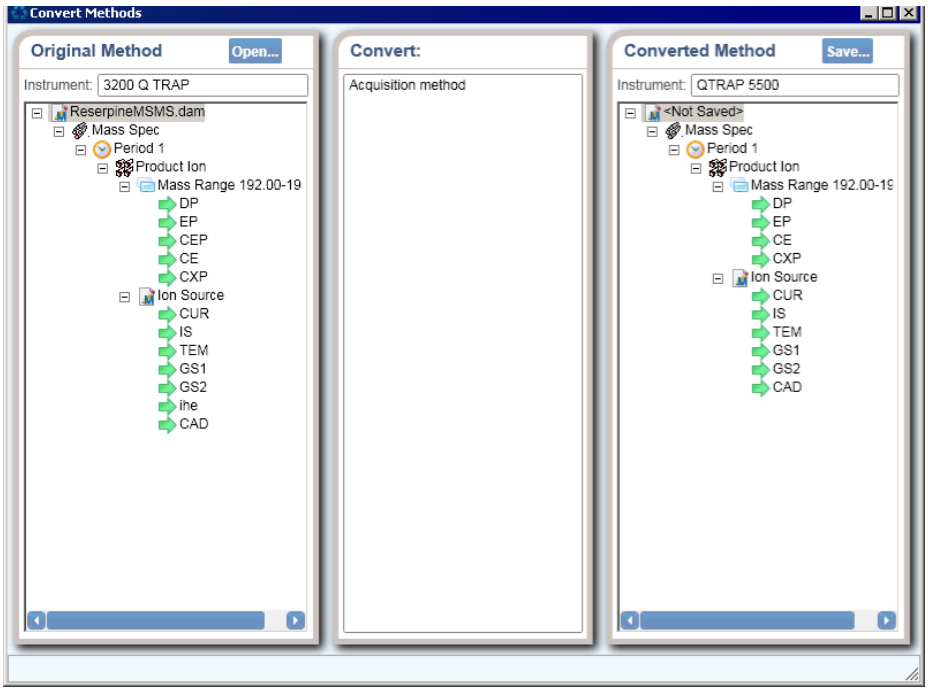


Figure 1-3 Convert Methods dialog

2. Click **Open**, navigate to the method that you want to convert, and then click **Open**.

   The Method Converter dialog displays the instrument name of the original method.

3. Click **Save**, type a name for the converted method, and then click **Save**.

Creating Quantitation Methods and Text Files

The Create Text File From Quan Method script exports a quantitation method to a tab-delimited text file. The Create Quan Method From Text Files script imports the information contained in a tab-delimited text file to a Quantitation Method File (.qmf). Currently, the Quantitation Method component in the Analyst® software does not support this functionality.

The Create Text File from Quan Method script creates a text file representation of a quantitation method file. A column in the text file is created only for all of the required fields. The optional fields will be created if the field value is not the same for all peaks in the quantitation method.

The Create Quan Method From Text Files script specifies the default values for any of the non-required fields in the text file such as integration algorithm or regression parameters. For more information, see Text File Format on page 17.

To use the Create Quan Methods From Text Files script

1. Click **Script** > **Create Quan Methods From Text Files**.

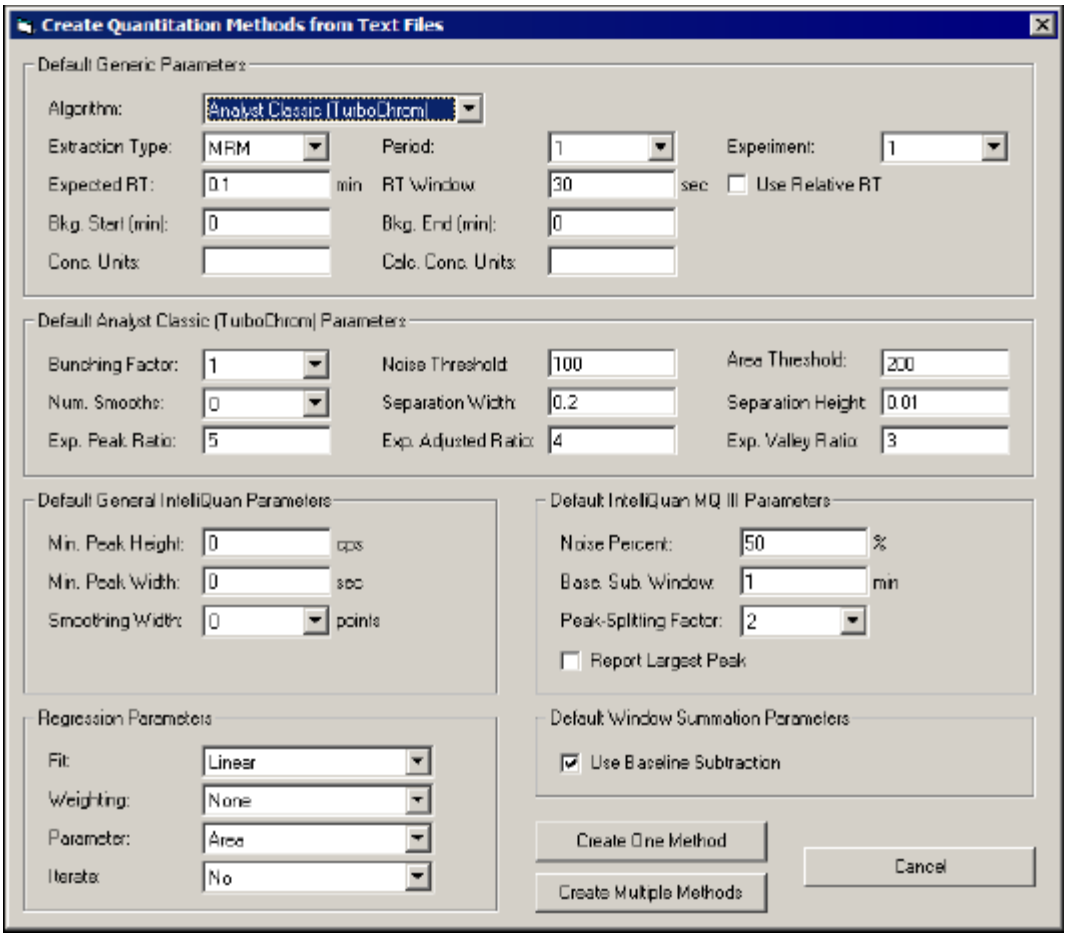


Figure 1-4 Create Quantitation Methods from Text Files dialog

2. Use the parameters in the **Default Generic Parameters** group to create a quantitation method. The **Algorithm**, **Extraction Type**, **Period**, and **Experiment** fields are not available in the Analyst software. Set the following parameters as required:
   - In the **Algorithm** drop-down list, select a peak-finding algorithm. The Window Summation algorithm sums all the intensities in the retention threshold and will not find any peaks.
   - In the **Extraction Type** drop-down list, select the type of data that will be integrated.
   - In the **Period** and **Experiment** drop-down lists, select the period number and experiment number.

   The Default Analyst Classic Parameters, Default General IntelliQuan Parameters, Default IntelliQuan MQ III Parameters, and the Default Window Summation Parameters groups contain the parameters that are used by the algorithm selected in the Algorithm field.

   Note: The Smoothing Width field in the Default General IntelliQuan Parameters group is half the smoothing width.

3. Select the **Use Baseline Subtraction** check box to have the Window Summation algorithm sum the intensities to the horizontal line at the minimum intensity of the data points within the summation window, as opposed to summing down to the intensity zero.
4. In the **Regression Parameters** group, select the regression information. The information specified here is applied to every analyte peak. Unlike the previous parameters, it is not possible to indicate this information in the text files; therefore, the same regression parameters are applied to all analytes. For a full description of the parameters, see the Help.
5. To create one quantitation method, click **Create One Method** and then navigate to the text file that will be used to create the quantitation method.
6. To create multiple methods from multiple text files, click **Create Multiple Methods** and then navigate to the folder. A quantitation method is created for each text file in this folder.

To use the Create Text File from Quan Method script

1. Create and save a quantitation method in the Analyst software.
2. Click **Script** > **Create Text File from Quan Method**.

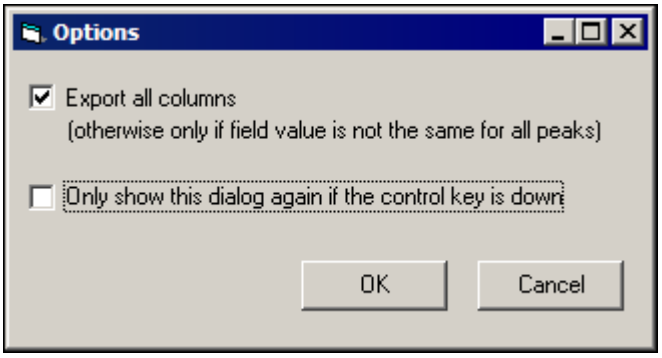

Options
Export all columns
(otherwise only if field value is not the same for all peaks)
Only show this dialog again if the control key is down
OK
Cancel

Figure 1-5 Options dialog

3. Select the **Export all columns** check box and then click **OK**.
4. Navigate to and select the quantitation method file (.qmf).
5. Navigate to and select the location of the text file.

   The script will generate the text file.

Text File Format

The text files used to create the quantitation methods (Create Quan Methods from Text Files) and generated from the methods (Create Text File from Quan Method) are in the following format:

- Separate the various fields with tab characters and end each line with a carriage return / line feed characters.
- The very first row of the file should contain column headings. All of the columns shown in the following table marked as Required must be present; the remaining columns are optional. The actual order of the columns is not important.
- Each subsequent line should contain the information as shown in the table for either one analyte or an internal standard peak.

Table 1-1 Text File Formats

Column name	Required	Description
Peak Name	Yes	The name of the analyte or internal standard peak.
First Mass	Yes	For MRM data, the Q1 mass for the peak; for full-scan data, the starting mass for the XIC to integrate; for Q1 MI or Q3 MI data, the mass.
Second Mass	Maybe	This field is required when integrating full-scan or MRM data, but not for Q1 MI or Q3 MI data. For MRM data, this is the Q3 mass for the peak; for full-scan data, it is the ending mass for the XIC to integrate.
Extraction Type	No	The type of data to integrate. If present, this should be one of: 0 – MRM data 1 – Q1 MI or Q3 MI data 2 – full-scan data

Table 1-1 Text File Formats (Continued)

Column name	Required	Description
Is IS	No	Specifies whether the current peak is an internal standard or an analyte. Select Yes if the peak is an internal standard; otherwise select No. If this column is not present, then all peaks defined are assumed to be analytes. Note: Internal standard peaks should be defined first in the text file before any analyte peaks that use that IS.
IS Name	No	For analyte peaks, specifies the name of the corresponding internal standard (if any). If a given analyte will not use an internal standard, leave the contents of this field empty. For internal standard peaks themselves, the contents of this field are ignored.
Period	No	The period number for the peak (from 1 to the number of periods in the data).
Experiment	No	The experiment number for the peak (from 1 to the maximum number of experiments in the period).
Use Relative RT	No	For analyte peaks that are using an internal standard, specifies whether or not the expected retention time is relative to that of the IS. Select Yes if so; otherwise, select No. The contents of this field are ignored for other peaks, but must still contain either Yes or No.
Conc Units	No	The concentration units.
Calc Conc Units	No	The calculated concentration units.
Bkg Start	No	Start time in minutes for the peak background. Note that this parameter does not affect the peak integration in any way, however it does affect how the noise (and hence S/N) is calculated.
Bkg End	No	End time in minutes for the peak background.
Expected RT	No	The expected retention time in minutes (from 0 to 1666).
RT Window	No	The retention time window in seconds (from 1 to 1000).
Algorithm	No	Specifies which peak-finding and integration algorithm should be used. If present, this should be one of: 0 – Analyst Classic (TurboChrom) 1 – IntelliQuan - IQA II (Automatic) 2 – IntelliQuan – MQ III 3 – Window Summation Note that the window summation algorithm is not available from within the Analyst software user interface, but can be enabled using the script.
Bunching Factor	No	The bunching factor for the peak (from 1 to 100) when using the TurboChrom algorithm.
Num Smooths	No	The number of smooths (from 0 to 10) when using the TurboChrom algorithm.

Table 1-1 Text File Formats (Continued)

Column name	Required	Description
Noise Threshold	No	The noise threshold (from 1^{-6} to 1^{9}) when using the TurboChrom algorithm.
Area Threshold	No	The area threshold (from 1^{-6} to 1^{12}) when using the TurboChrom algorithm.
Separation Width	No	The separation width (from 0 to 5) when using the TurboChrom algorithm.
Separation Height	No	The separation height (from 0 to 1) when using the TurboChrom algorithm.
Exp Peak Ratio	No	The exponential peak ratio (from 1 to 1^{6}) when using the TurboChrom algorithm.
Exp Adjusted Ratio	No	The exponential adjusted ratio (from 2 to 1^{6}) when using the TurboChrom algorithm.
Exp Valley Ratio	No	The exponential valley ratio (from 1 to 1^{6}) when using the TurboChrom algorithm.
Min Height	No	The minimum allowed peak height (from 0 to 1^{16}) when using the IntelliQuan algorithm.
Min Width	No	The minimum allowed peak width (from 0 to 1^{16}) in seconds when using the IntelliQuan algorithm.
Smooth Width	No	The half-width of the Savitzky-Golay smoothing filter (from 0 to 20) when using the IntelliQuan algorithm.
MQ III Noise Percent	No	The noise percentage when using the MQ III option of the IntelliQuan algorithm. This should be an integer from 0 to 100.
MQ III Baseline Sub Window	No	The baseline subtraction window (from 0 to 10 minutes) when using the MQ III option of the IntelliQuan algorithm.
MQ III Peak Splitting Factor	No	The peak-splitting factor (from 0 to 10) when using the MQ III option of the IntelliQuan algorithm.
MQ III Use Largest	No	When using the MQ III option of the IntelliQuan algorithm, specifies whether the largest peak (within the retention time window) or the peak whose retention time is closest to that expected is reported. Select Yes to use the largest peak and No to use the closest.
Summation Baseline Sub	No	When using the special window summation algorithm, specifies whether the area should be integrated to the intensity=0 line or to the intensity value of the least intense data point within the window.

The following table shows an example text file for full-scan data. Imagine that there are tabs between the columns and a hard return at the end of each line.

Table 1-2 Example Text File for Full-Scan Data

Peak Name	First Mass	Second Mass	Bunching Factor
Analyte Peak 1	500.10	500.70	1

Table 1-2 Example Text File for Full-Scan Data (Continued)

Peak Name	First Mass	Second Mass	Bunching Factor
Analyte Peak 2	812.00	813.00	2
Analyte Peak 3	400.00	401.00	3

The following table shows one more example for MRM data. The Analyte Peak 1 will be set up to use the specified internal standard and Analyte Peak 2 will not use an internal standard.

Table 1-3 Example Text File for MRM Data

Peak Name	Is IS	IS Name	First Mass	Second Mass
IS Peak 1	Yes		500.10	413.20
Analyte Peak 1	No	IS Peak 1	600.20	382.10
Analyte Peak 2	No		400.00	312.1

The following table contains a mixture of full-scan and MRM data in different experiments:

Table 1-4 Example Text File for MRM Data

Peak Name	Extraction Type	Experiment	First Mass	Second Mass
Analyte Peak 1	0	1	500.10	413.20
Analyte Peak 2	0	1	600.20	382.10
Analyte Peak 3	2	2	812.00	813.00
Analyte Peak 4	2	2	400.00	401.00

DBS Settings

The DBS (Dynamic Background Subtraction™ algorithm) is a feature that ensures better selection of precursor ions in an IDA (Information Dependant Acquisition) experiment. When DBS is activated, IDA uses a spectrum that has been background subtracted to select the candidate ion of interest for MS/MS analysis, as opposed to selecting the precursor from the survey spectrum directly. DBS enables detection of species as their signal increases in intensity, thus focusing on detection and analysis of the precursor ions on the rising portion of the LC peak, up to the top of the LC peaks (maximum intensity).

The DBS functionality is embedded in the Analyst® 1.6 software for IDA experiments; however, the associated parameters are not accessible in the Analyst software. The hidden parameters and their default values are as follows:

- Average number of previous spectra = 4
- Smooth before subtract: activated
- Smooth = 5 data points.

Use this script to change the default parameters to ones that are more representative of the experimental conditions. Depending on the cycle time and chromatography, the default settings may result in an obvious candidate ion from being omitted for dependent MS/MS analysis or the same candidate ion may be selected for MS/MS analysis over the entire LC peak. Therefore, this script will be useful to customers who find that the embedded default values are not appropriate for their analysis.

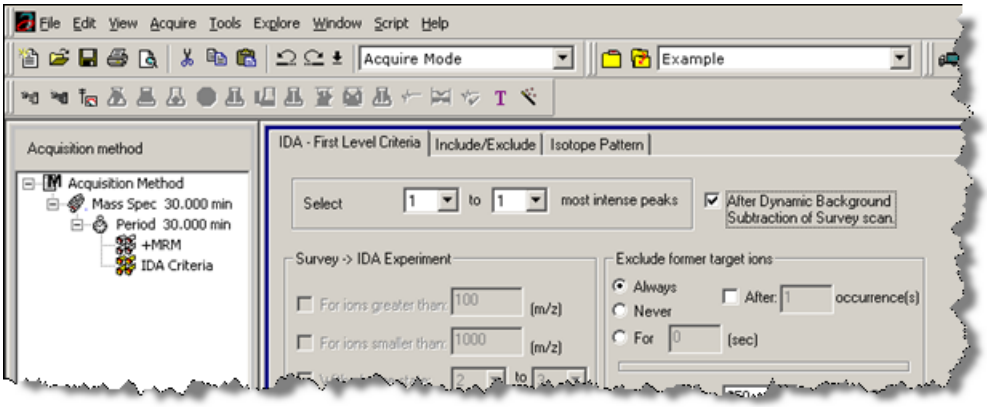
After the script is installed, the DBS feature uses the settings in the script. The script will remember the last settings used.

Prerequisites

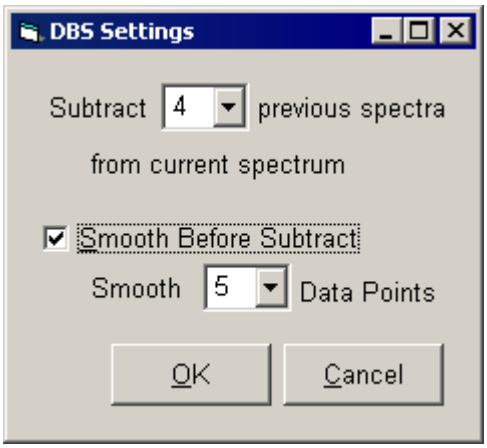
- Analyst 1.6 Software installed
- Administrator rights on the computer

To use the script

1. Activate the DBS feature by selecting the **After Dynamic Background Subtraction of Survey scan** check box on the **IDA – First Level Criteria** tab.



2. Although the DBS feature is activated at the method level, the DBS options are set using the script. From the Analyst software menu, click **Script** > **DBS Settings**.



- **Subtract the _ previous spectra from current spectrum**: Use this field to select the number of spectra averaged to represent the background signal. These spectra are taken immediately before the current survey spectrum. Typical values used are between 2 and 5.
- **Smooth Before Subtract**: Select to make sure that the current survey spectrum is smoothed using a Savitzky-Golay smooth before the subtraction step. Select the number of points to consider in the process. A typical value is 5.

Survey Scans Supported

The DBS feature can be used with any IDA survey scans currently supported: EMS, EMC, Precursor Scan, Neutral Loss, MRM, Q3 MS and Q3 MI. DBS does not apply to the confirmation or dependant levels.

Known Limitations and Issues

- With the exception of DBS being on or off, DBS parameters are not stored as part of the file information. In order to recall which settings were used, the user should document the parameters selected during the acquisition.
- DBS is meant for use during LC analysis, therefore, when performing IDA by infusion it is recommended to have DBS deactivated.

Define Custom Elements

Use this script to select a custom isotope pattern when working with radio-labeled compounds. An experiment-specific element pattern is used in the data interpretation in conjunction with the Analyst® software calculators or the Metabolite ID application.

The custom isotope patterns are stored together with the information from the periodic table elements in the element definition file, SAElements.ini, which is located in the Analyst\bin folder. In the element definition file, the custom elements must have a unique symbol and an atomic number of 104 or higher.

Prerequisites

The following program is optional:

- Metabolite ID 1.3 for Analyst QS 2.0 software

When the script is launched for the first time, a backup copy of the SAElements.ini file is saved in the API Instrument folder. If needed, the edited SAElements.ini file in Analyst\bin folder can be replaced with this file.

To update the element definition file successfully

1. Do not open the SAElements.ini file in another text editor program while using the software.
2. Make sure the file access must be set to read/write.

To edit the Define Custom Elements table

The custom element table cannot be edited in the dialog. After the element definition file is updated, the custom elements can be used with the Analyst software calculators.

1. Click **Script** > **DefineCustomEl**.

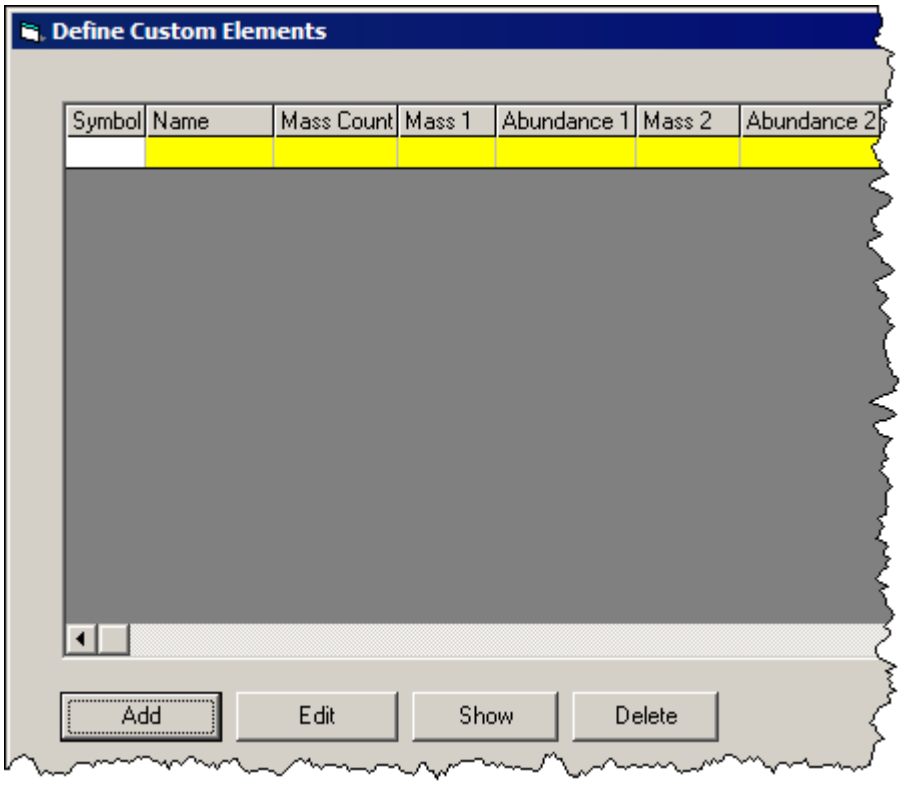


Figure 1-6 Define Custom Elements dialog

2. In the table, click the row containing the element.

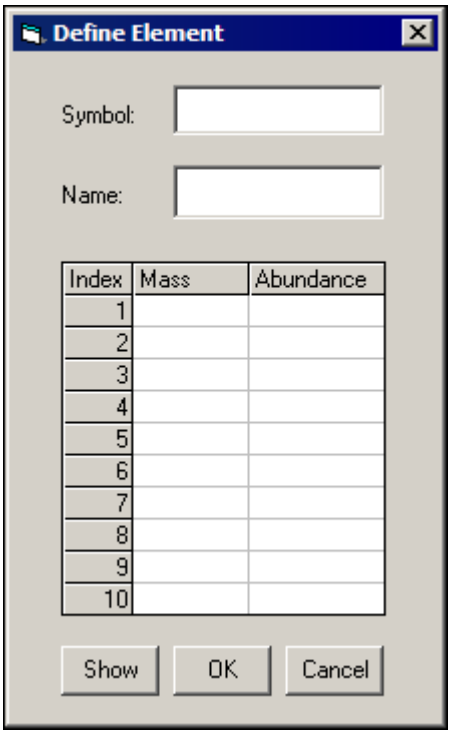


Figure 1-7 Define Element dialog

3. Edit the fields and then click **OK**.
4. To save the updated element definition file and exit the program, click **OK**.

To view the custom element symbol, custom element name, and custom pattern

- To view the custom pattern in the mass/relative intensity graph, in the **Define Custom Elements** dialog, click **Show**.

  The Isotopic Distribution dialog appears.

  The total of the individual isotope abundance for an element stored in the element definition file must be equal to one. Therefore, the abundances entered in the Define Element Window are rescaled before they are added to the Define Custom Elements dialog. This Isotopic Distribution dialog cannot be edited. You can zoom in the area of interest by dragging along the corresponding x- or y-axis region.

  The application requires the gen01.wiff example file to display custom pattern. If the gen01.wiff file is not in the Example folder in the Analyst Data\Projects folder, you will be prompted to find this file.

Delete Others

Use this processing script to delete all panes except for the active one.

To use the script

1. With a sample file (.wiff) with multiple panes open, click a pane.
   The pane becomes the active pane.
2. Click **Script** > **DeleteOthers**.
   All the panes except for the active one are deleted.

DFT Tracker

The Dynamic Fill Time (DFT) Tracker script tracks the DFT settings used during QTRAP® instrument scans. You can use the script to determine the optimal fill time for linear ion trap (LIT) mode to obtain high data quality over a wide dynamic range. The DFT Tracker monitors the following LIT scan types: Enhanced MS (EMS), Enhanced Resolution (ER), Enhanced Product Ion (EPI), and MS/MS/MS (MS3).

To use the script

- Click **Script** > **DFTTracker**.

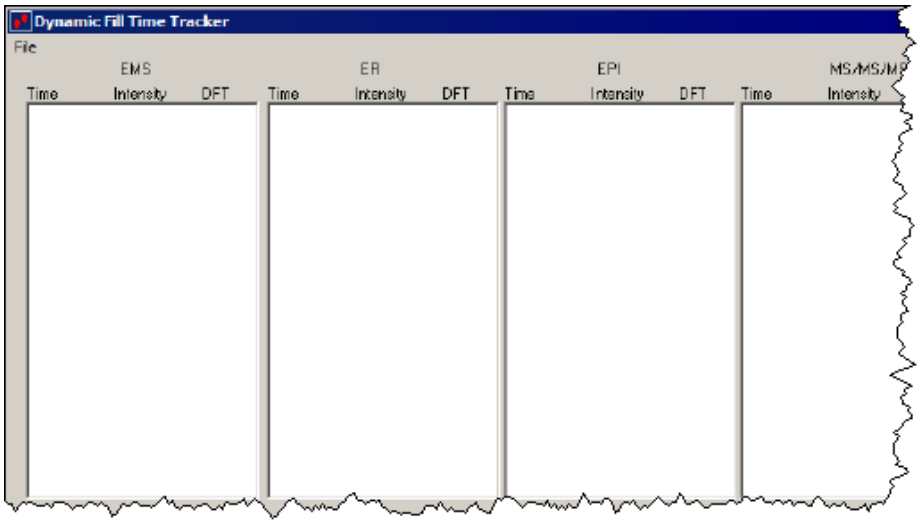


Figure 1-8 Dynamic Fill Time Tracker dialog

DFT Tracker monitors the dynamic changes occurring during a real-time run.

The system dynamically calculates the time required to fill the linear ion trap. For abundant compounds, a short fill time reduces the space charge effects by limiting the number of ions in the ion trap; on the other hand the longer fill time increases weak signals by allowing the ions to accumulate.

Export IDA Spectra

Use this script to export data in a format that can be searched using a third-party application. The Export IDA Spectra script exports every dependent product spectrum from an IDA (Information Dependent Acquisition) LC/MS run to a series of text files. These text files can then be submitted and searched using Sequest. The export is optimized so that any spectra in adjacent cycles with the same precursor *m/z* are combined into a single spectrum. This optimization also applies to spectra with the same precursor but which reside in different experiments, most likely using different values of the collision energy.

The charge state of the precursor ion is a required input. Sequest tries to automatically determine this from the isotope spacing at the precursor *m/z* in the IDA survey spectrum. Note that while this determination is usually correct, it is not always so.

To use the script

It is assumed that the first experiment of the IDA method represents the survey spectrum and that all other experiments represent dependent product spectra. Therefore, this script cannot be used if there are multiple survey experiments.

1. With an IDA chromatogram in an active pane, click **Script** > **Export IDA Spectra**.

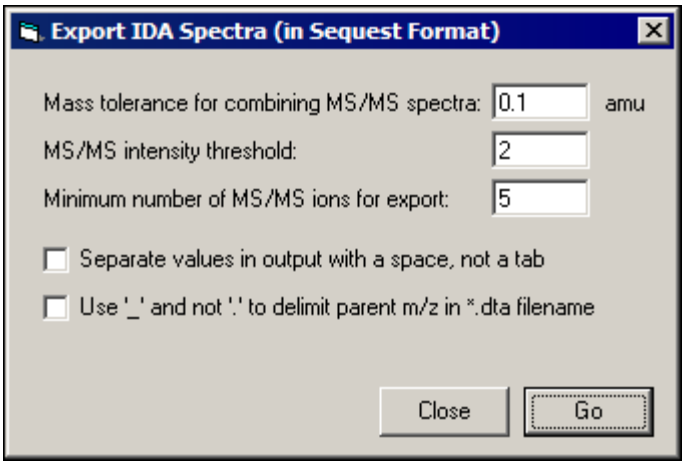

Export IDA Spectra (in Sequest Format)
Mass tolerance for combining MS/MS spectra:
0.1
amu
MS/MS intensity threshold:
2
Minimum number of MS/MS ions for export:
5
Separate values in output with a space, not a tab
Use '_' and not '.' to delimit parent m/z in *.dta filename
Close
Go

Figure 1-9 Export IDA Spectra (in Sequest Format) dialog

2. In the **Mass tolerance for combining MS/MS spectra** field, type the tolerance to be used to determine if two precursor *m/z* values should be considered identical. If the precursors for two sequential product spectra differ by less than this value, the spectra are added and a single text file is exported.
3. In the **MS/MS intensity threshold** field, type the threshold that is applied to each product spectrum after it is centroided. It is assumed that peaks below this threshold are most likely noise. Type 0 in the field if you do not want to use a threshold.
4. In the **Minimum number of MS/MS ions for export** field, type the minimum number of ions that must be present in a product spectrum, after centroiding and thresholding, in order for a text file to be exported. If a spectrum does not contain the specified number of ions, it is assumed that the quality of the spectrum is too low to merit exporting.

5. To separate fields in the output files with a space character, select the **Separate values in output with a space, not a tab** check box; otherwise a tab character is used. (Certain versions of Sequest require a space delimiter.)
6. To export the text files, click **Go**.
7. In the **Save As** dialog, type a location and root file name for the exported text files.

   Before being exported to a text file, each of the product spectra is centroided. The cycle number range and charge state is appended to this file for each exported spectrum.

Export Sample Information

Use this script to extract sample information, such as the name, sample ID, comment, and acquisition method name for all samples in the .wiff file. You can define the information you would like exported, and the script saves the information in an .inf file located in the same folder as the .wiff file.

To use the script

1. With a chromatogram or spectrum in an active pane, click **Script** > **ExportSampleInformationFromMultipleSampleinOneWiff** while pressing the Ctrl key.

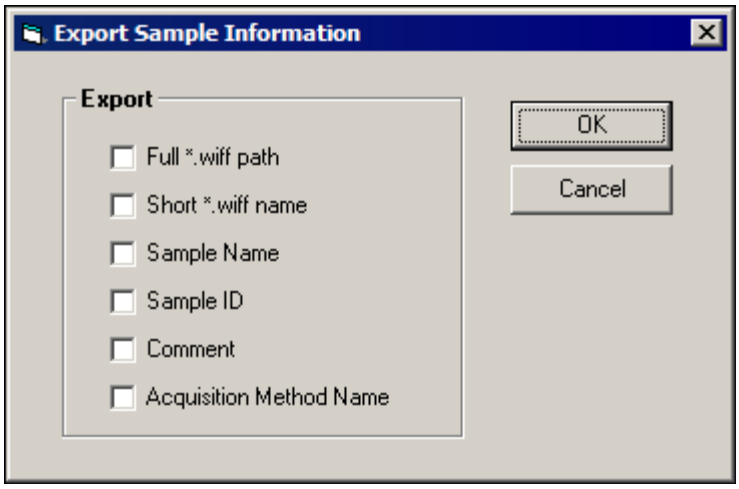


Figure 1-10 Export Sample Information dialog

2. Select the check boxes that correspond to the information that you want to export and then click **OK**.

Export to JCamp

You can use the Analyst® software to export graph data to a tab-delimited text file that can be read by most applications; however, some applications require a more specific format.

With the Export to JCAMP script, you can export graph data in the JCAMP format. The script works on both chromatograms and spectra. For chromatograms, depending on the number of selections made, either all the spectral data of the chromatogram is exported, or the averaged sum of the selected regions is exported. If you are using this script on a single spectrum, then only that data is exported. This script can also be attached to a batch so that the export occurs automatically after the sample is acquired.

Table 1-5 shows an overview of the operation of the script. When run interactively the exact behavior depends on the active Analyst software data.

Table 1-5 Script Operation

Modes	Active data	Operation
Interactive	Spectrum	You will be prompted for the name of the JCamp file and the active spectrum exported to it.
Interactive	Chromatogram with two or more selections	You will be prompted for the name of the JCamp file and an averaged spectrum corresponding to each of the chromatogram's selections exported to it.
Interactive	Chromatogram with one or no selections	You will be prompted for the name of the JCamp file and every spectrum for the run exported to it.
Batch	N / A	The name of the JCamp file is generated by appending the sample number to the name of the .wiff file and changing the extension to jdx. Every spectrum for the run is exported to the JCamp file. For multiple period/experiment data, a separate file is exported for each experiment (the period and experiment numbers are appended to the filename).

To use the script

1. To use the Export to JCAMP do one of the following:
   - With either a chromatogram or a spectrum in an active pane, click **Script** > **Export to JCAMP**.
   - In the **Batch Editor** dialog, type Analyst Data \API Instrument\Processing Scripts\Export to Jcamp.dll in the **Batch Script** field.

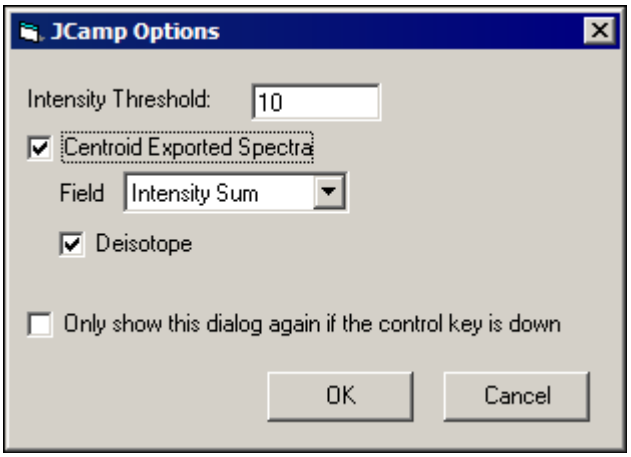


Figure 1-11 JCAMP Options dialog

2. To select the centroiding options, do one of the following:
   - Check the **Centroid Exported Spectra** check box to centroid the spectra before exporting to the JCAMP format.
   - To have the JCAMP Options window appear only if the Ctrl key is pressed when clicking the script from the Script menu, or when submitting the batch to the queue, select the **Only show this dialog again if the control key is down** check box.
3. To continue processing and to have the spectrums exported, click **OK**.
4. When prompted, type the file name of the exported JCAMP file. When a script is attached to the batch, the file name is automatically generated using the following format: [WiffFileName]_[Sample#]_[Period#]_[Experiment#].jdx

IDA Trace Extractor

Use this script to review the survey data collected using IDA (Information Dependent Acquisition) based on the information in the corresponding dependent data. The script searches the MS/MS data for given neutral losses or fragments and then calculates the Extracted Ion Chromatograms (XICs) for the precursor masses, which give the specified losses or fragments. The XICs are overlaid in Explore mode and their peaks are labeled with the precursor mass (Figure 1-12).

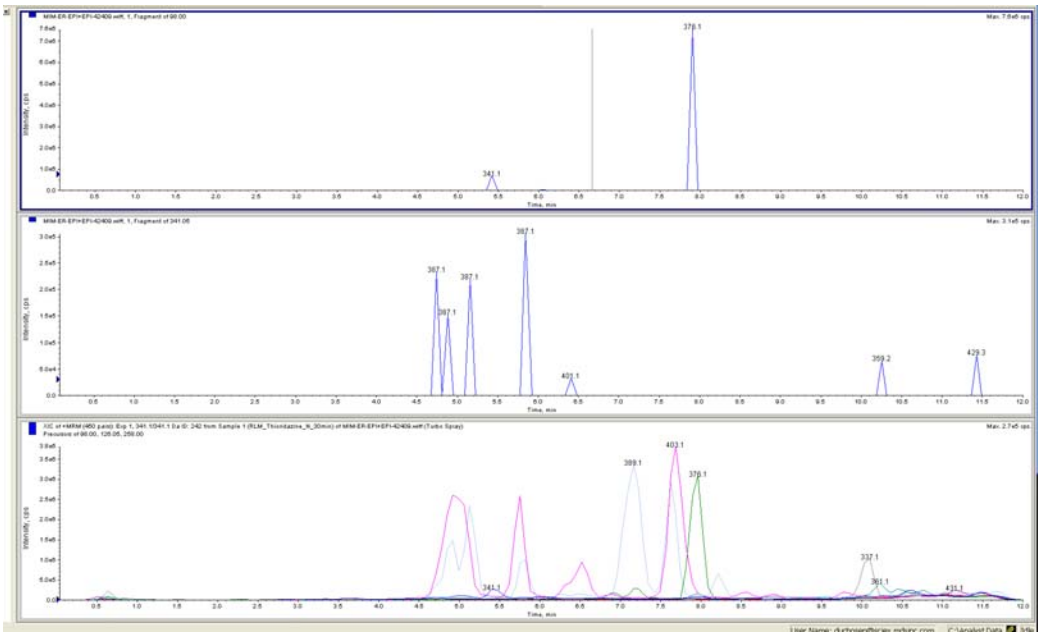


Figure 1-12 Characteristic Traces in Dependent Experiment and XICs of the Survey Experiment. The characteristic *m/z* 387.1 detected in negative mode was converted to *m/z* 389.1 in the positive survey scan.

You can use the script to do the following:

- Specify a list of expected fragments or neutral losses, in either the positive mode polarity, the negative mode polarity or both, in terms of fragment formula or mass.
- Save the list of masses and fragments for a compound class and load them to the settings at a later time.
- Process just a selected time region in the chromatogram.
- Display precursor (survey scan) or fragment (dependent scan) XIC trace that yield given fragment or neutral loss.
    - From the survey scan chromatograms the user can link to any survey scan spectra
    - From the dependent scan traces the user can link to any dependent scan spectra
- Reduce the precursor XIC traces to show just peaks that have corresponding neutral losses (or fragments)
- Save the precursor mass list in a text file for further processing; build the list of precursors from a set of samples
- Save the list of masses in a format that can be loaded to the XICfromTable script

- Save the list of masses and peaks in a format that can be loaded to the CreateQuantMethodFromText script.

Note: The script is compatible with MRM / MIM IDA data and with the Analyst® 1.6 software.

Note: The script supports parallel data processing from positive and negative experiments. Multiple survey and dependent experiments of any polarity can be used.

To use the script

Note: The Analyst software version for a specific file can be displayed in the file properties > comments.

1. With a chromatogram of IDA data open in an active pane, click **Script > IDATraceExtractor**.

   You can process a full chromatogram or just a selected region (make a selection before running the script).

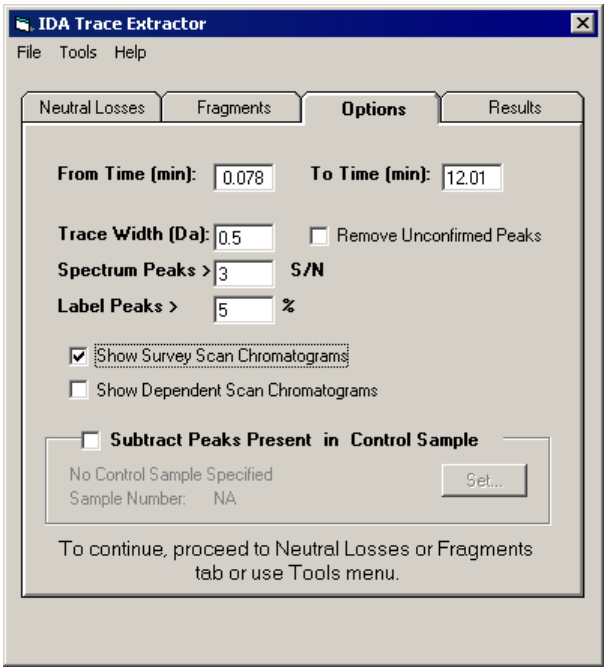


Figure 1-13 IDA Trace Extractor dialog

2. In the **Options** tab of the script, set the fields as required. For more information, see Table 1-6 *Tab and Menu Parameters* on page 36.
3. To store the retention times and precursor masses found during processing, click the **Results** tab, type a filename, and then select **Save Precursor Information**.

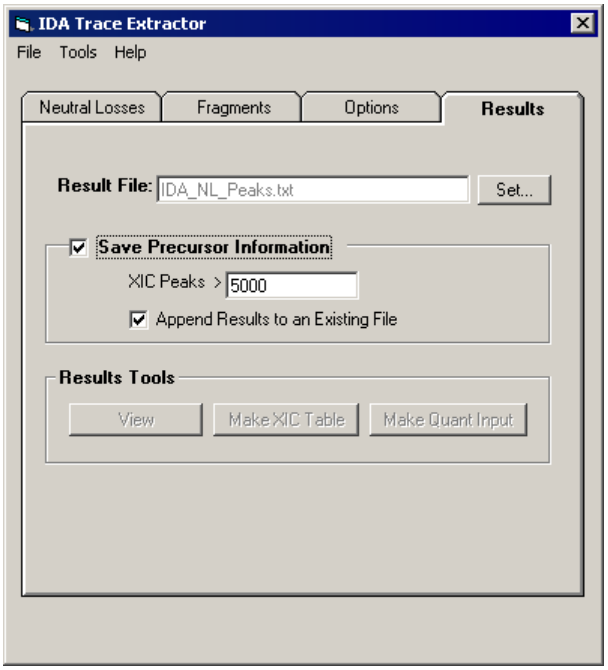


Figure 1-14 IDA Trace Extractor dialog: Results tab

4. To review or enter the mass information, click the **Neutral Losses** or **Fragments** tab. Type the neutral losses and fragments as masses or chemical formulas. You can also specify the polarity of the Neutral Loss or fragment spectrum experiment where the specified neutral loss or fragment is expected to be found.

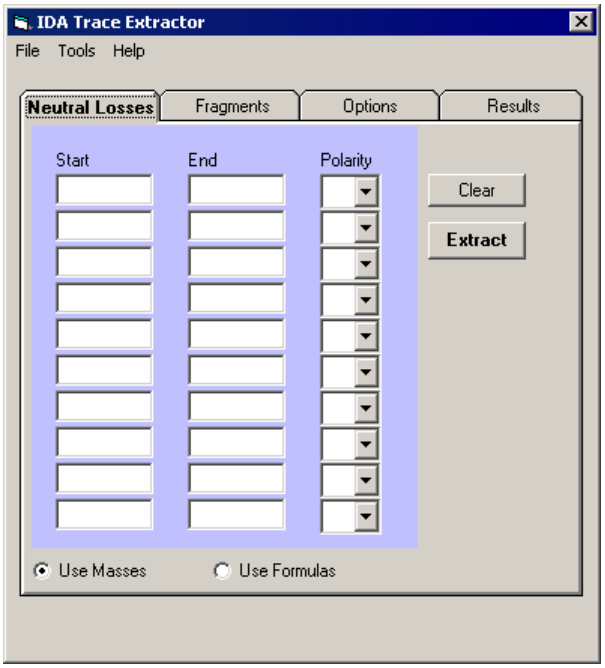


Figure 1-15 IDA Trace Extractor dialog: Neutral Losses tab

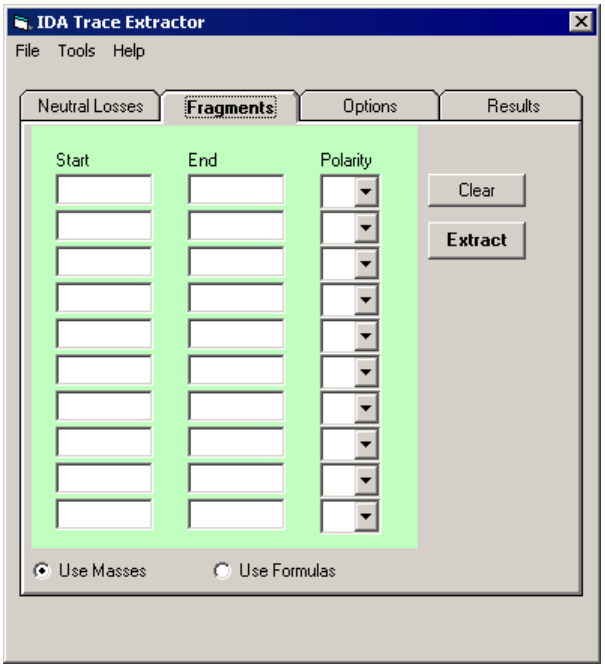


Figure 1-16 IDA Trace Extractor dialog: Fragments tab

5. Click **Extract** to find the survey XIC traces that give the selected neutral loss or fragment.
6. If the precursor information was saved, the found precursor mass/time data can be converted to a compatible format using other scripts. To convert the data, click the **Results** tab and then select the Results File. View and edit this file as required.
   - To make a format for the XICfromTable script, click **Make XIC Table**. The Results File will be converted into a file of the same name with the suffix _XIC.
   - To make a format for the **CreateQuantMethodFromText** script on the **Precursor XICs** dialog, click **Make Quant Input**. The Results File will be converted into a file of the same name with the suffix _Peaks.
7. Click **File** > **Save Settings as**. Alternatively, previously saved settings can be used. Click **File** > **Load Settings** to open previously saved settings.

Several functions are available in the Tools menu. You can start processing without switching to a specific tab.

Table 1-6 Tab and Menu Parameters

Location	Parameters	Description
Neutral Losses	Use Masses	Select the required neutral loss(es) as mass.
Neutral Losses	Use Formulas	Select the required neutral loss(es) as formula.
Neutral Losses	Start	Low mass limit (from mass) for the neutral loss(es).
Neutral Losses	End	High mass limit (to mass) for the neutral loss(es).

Table 1-6 Tab and Menu Parameters (Continued)

Location	Parameters	Description
Neutral Losses	Formula	Chemical formula of the neutral loss.
Neutral Losses	Extract	Start data processing according to the current settings.
Neutral Losses	Clear	Clear all neutral losses in the settings.
Neutral Losses	Polarity	Select the polarity of the Neutral Loss experiment where the specified neutral loss is expected to be found.
Fragments	Use Masses	Describe the characteristic fragment(s) in terms of their *m/z*.
Fragments	Use Formulas	Describe the characteristic fragment(s) in terms of their formulas.
Fragments	Start	Low mass limit (from mass) for the fragment *m/z* window.
Fragments	End	High mass limit (to mass) for the fragment *m/z* window.
Fragments	Formula	Chemical formula of the fragment (protonated/ deprotonated form).
Fragments	Extract	Start data processing according to the current settings.
Fragments	Polarity	Select the polarity of the fragment spectrum experiment where the specified fragment is expected to be found.
Fragments	Clear	Clear all fragments in the settings.
Options	From Time (min)	Start of time region to be processed.
Options	To Time (min)	End of time region to be processed.
Options	Trace Width (Da)	Width of XIC traces in resulting survey scan and dependent scan chromatograms and mass tolerance window for processing in case that the neutral losses or fragments are specified as chemical formulas.
Options	Remove Unconfirmed Peaks	Review all peaks in the survey XIC traces and retain only those that were validated based on the data in corresponding dependent scan.
Options	Spectrum Peaks >	Minimum size of diagnostic peak in dependent scan in terms of signal to noise (lowest measurable signal).
Options	Label Peaks >	Peak label threshold applied to resulting survey and dependent scan chromatograms (note that just the largest peak in each trace is labeled).

Table 1-6 Tab and Menu Parameters (Continued)

Location	Parameters	Description
Options	Show Survey Scan Chromatograms	Display XICs (original or filtered) from the survey scan that correspond to parent masses yielding specified fragment or neutral loss.
Options	Show Dependent Chromatograms	Display neutral loss traces (one for each neutral loss) reconstructed from dependent scan data.
Options	Subtract Peaks Present in Control Sample	Remove peaks that can be found in the control sample XICs from the survey scan chromatograms.
Results	Result File	Select Results file (containing identified peak times and masses will be saved).
Results	Save Precursor Information	Write processing results (list of found peaks - times and masses) to selected results file.
Results	XIC Peaks >	Minimum size of the peak in survey scan to be stored in the results file.
Results	Append Results to an Existing File	Do not overwrite existing results file.
Results	View	Open the selected results file.
Results	Make XIC Table	Use the selected results file to prepare settings file for XIC from Table script.
Results	Make Quant Input	Use the selected results file to prepare an input for CreateQuanMethodFromText script.
File Menu	Load Settings…	Load previously saved script settings to the interface.
File Menu	Save Settings As…	Save current script settings for future use.
File Menu	Set Results File…	See Results/Result File
Tools Menu	Extract Fragments	See Fragments/Extract
Tools Menu	Extract Neutral Losses	See Neutral Losses/Extract
Tools Menu	Clear Fragments	See Fragments/Clear
Tools Menu	Clear Neutral Losses	See Neutral Losses/Clear
Tools Menu	Make XIC Table	See Results/Make XIC Table
Tools Menu	Make Quant Input	See Results/Make Quant Input

Table 1-7 Related Scripts

Script name	Description
Export to JCamp	Converts spectra from .wiff format to JCamp format.

Table 1-7 Related Scripts (Continued)

Script name	Description
MSMS Methods from MW Lists	Allows lists of molecular weights obtained from text files to be used as the basis for creating a series of MS/MS acquisition methods.
Multiple Batch Scripts Script	Allows multiple batch acquisition scripts to be used at the same time (the Batch Editor only allows a single batch script to be specified).
Unit Conversion	Converts from one set of concentration units to another.
Wiff To MatLab	Converts the data from a data file from the '.wiff' format to MatLab (.mat) format.

Label Selections

Use this script to add missing labels to the selected peaks in the active graph or to remove them.

The script can be run when a pane containing a spectrum or a chromatogram is active in Explore mode, and there are one or more selections in the active pane. If neither peak mass (spectrum) nor peak retention time (chromatogram) is available, the data will be marked with information for the selection maximum.

Note: Only font type and label color are synchronized with the automatic labels. For the best performance, synchronize the other font attributes manually in the Appearance Options dialog.

Note: Labeling spectra with centroid mass/charge state appends just the centroid mass. Labeling chromatograms with base peak ion mass or base peak ion intensity is not supported.

To use the script

- Do one of the following:
  - To add labels to selections in active graph, click **Script** > **LabelSelections**.
  - To see the script description and add the labels to an active Explore pane, hold the Shift key down while clicking the script.

Label XIC Traces

Use this script when a pane containing one or more XIC traces is active in Explore mode. There may be a time region selected in a trace. If there is no selection, a complete chromatogram will be considered for processing. The script labels the largest peak in each XIC trace with mass.

XIC traces with a maximum point of less than 5% of the most intense trace will not be labeled. Other types of traces (TIC, ADC) in the overlay will be ignored.

Note: No user settings are required for this script.

To use the script

- Do one of the following:
  - Click **Script** > **LabelXICs**.
  - To see the script description, and add the labels to an active Explore pane, hold the Shift key down while clicking the script.

Make Exclusion List from Spectrum

Use this script to create a text file containing all of the peaks from the currently active spectrum. The text file is in a format that can be directly imported into the Analyst® software IDA (Information Dependent Acquisition) exclusion list.

To use the script

If the spectrum has been previously manually centroided, the resulting peak list is exported directly to the exclusion text file. Otherwise, the script will first centroid the spectrum.

1. With a spectrum in an active pane, click **Script** > **Make Exclusion List from Spectrum**.

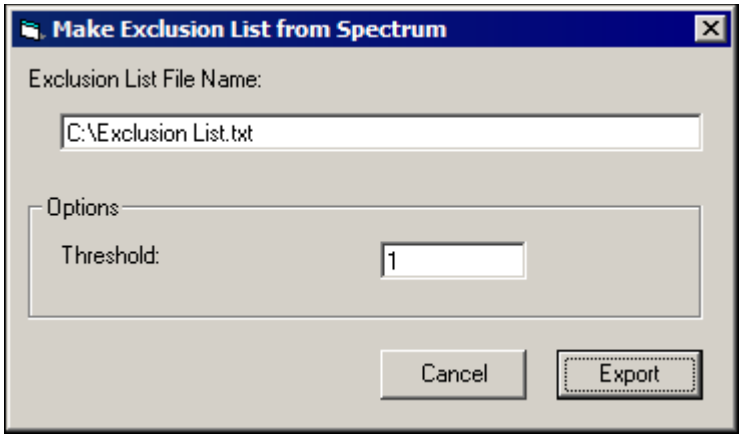


Figure 1-17 Make Exclusion List from Spectrum dialog

2. In the **Exclusion List File Name** field, type the name and path of the text file.
3. If required, in the **Threshold** field, type the threshold that will be applied to the centroided spectrum so **Threshold** field that small noise peaks are not included in the exclusion list. Type 0 in the to not use a threshold.
4. Click **Export** to export the exclusion list using the specified parameters.

Make Subset File

Use this script to manipulate data files by transferring samples to another data file, unpacking all samples in a data file to their own data file, and decomposing a single sample into many samples.

To use the script

Section 1: General

This section describes how to select a data file to work on.

1. Click **Script** > **MakeSubsetFile**.

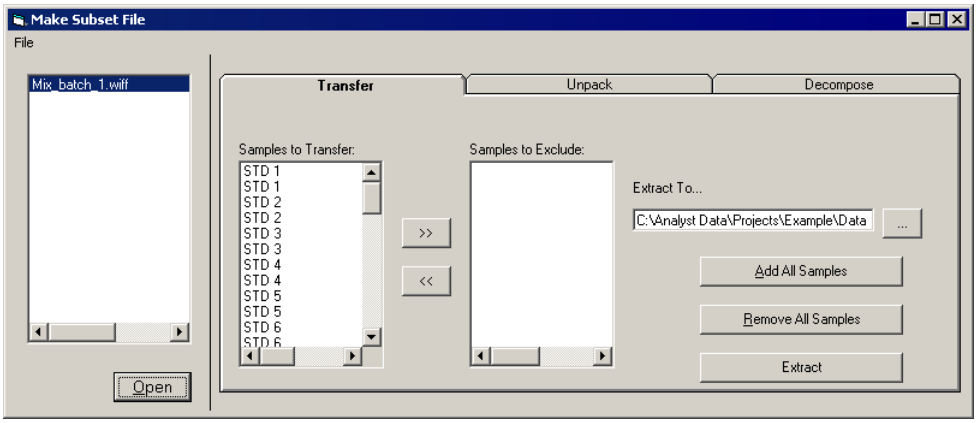


Figure 1-18 Make Subset File dialog

2. By default, all the files in the current project data folder are loaded. To add another file to this list, click **File** > **Open** and then navigate to the file.
3. Click the file.
4. To exit the program, click **File** > **Exit**.

Section 2: Transfer

This section describes how to transfer samples from one data file to another.

1. Click the **Transfer** tab.

   All the samples in the current working data file will automatically appear in the **Samples to Transfer** list.
2. To exclude a sample from the transfer, select it from the list and then click **>>**.

   The unwanted sample appears in the **Samples to Exclude** list.
3. To include an excluded sample in the transfer, select it from the **Samples to Exclude** list and then click **<<**.
4. To exclude all the samples, click the **Remove All** type the full path and the file name of the file in which the sample will extract, or navigate to a file. If the file does not exist, the Make Subset File script will create it.
5. To start the transfer, click **Extract**.

Section 3: Unpack

This section describes how to unpack every sample in one data file into its own data file.

1. Click the **Unpack** tab.
2. In the **Destination Directory** group, the location of the current working data file appears. Use this tree to select the location for the unpacked data files.
3. To create a new directory, right-click the directory tree. You will be prompted to type the new folder name and set the active folder.
4. In the **Output File Name text** field, type the output file name. Each sample unpacked from the working data file will begin with this name followed by the sample number in parentheses. Do not give an extension to this file (for example, do not include ".wiff"), because the Make Subset File program will automatically append this.
5. To begin unpacking the samples, click **Extract**.

Section 4: Decompose

This section describes how to decompose a sample into different samples.

1. Click the **Decompose** tab.
2. To use the Make Subset File script default values, select the **Use Defaults** check box.
3. To provide threshold values, deselect the **Use Defaults** check box and then type the values in the fields.
    - The **Noise** field contains the noise threshold value. This value indicates when a sub-sample begins and ends. If the intensity value exceeds this threshold, then it is considered a new sample. After an intensity value falls below this threshold, the sub-sample is considered complete and it is sent to the file.
    - The **Width** field contains the width threshold value. This value is used to prevent short periods of loud noise. If noise exceeds the noise threshold value for a short period of time (that is, have a small width) then without the width threshold this noise will be considered a new, sample. Only detected samples with a width that exceeds the width threshold will be exported to the file.
4. In the **Decompose To** field, type the full path and the file name of the file in which the sample will be decomposed, or navigate to a file. If this file does not exist, then the Make Subset File program will create it.
5. To start decomposing the sample, click **Extract**.

Manually Integrate

The Analyst® software has various peak-finding algorithms that determine and integrate peaks and then show the results in the peak list. If required, you can also use the Manually Integrate script to integrate a selected region of a graph because the software may not have detected the peak or perhaps because only a portion of the peak is of interest.

Use the script to draw a line on a chromatogram and have the area above the line integrated. The integrated area is highlighted in the chromatogram, and the calculated area of the region can be pasted on the graph. The results are displayed in the script window, which can also be added to the graph or exported to a text file for storage.

To use the script

1. With a chromatogram opened, click **Script** > **Manually Integrate**.

   The fields in the Result group are modifiable from the Options button.

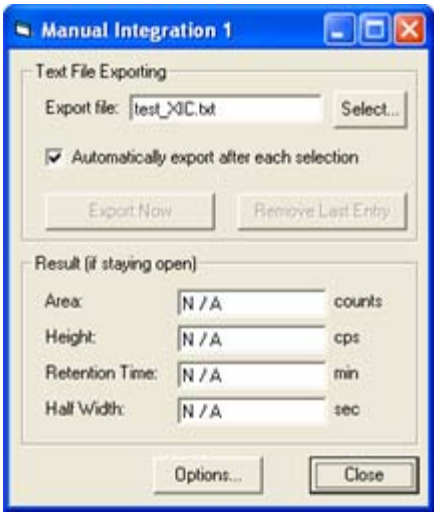


Figure 1-19 Manual Integration 1 dialog

2. In the **Text File Exporting** group, click **Select** to navigate **to a text file.**
3. Do one of the following:
   - Select **Automatically export after each selection** to automatically export the results to the specified text file.
   - Click **Export Now** to export the current results.
4. To remove the last exported results from the text file, click **Remove Last Entry**.
5. To populate the fields in the **Result** group, highlight a section of the graph. The values are calculated using the selected area of the graph.
6. To change the manual integration options, click **Options**.

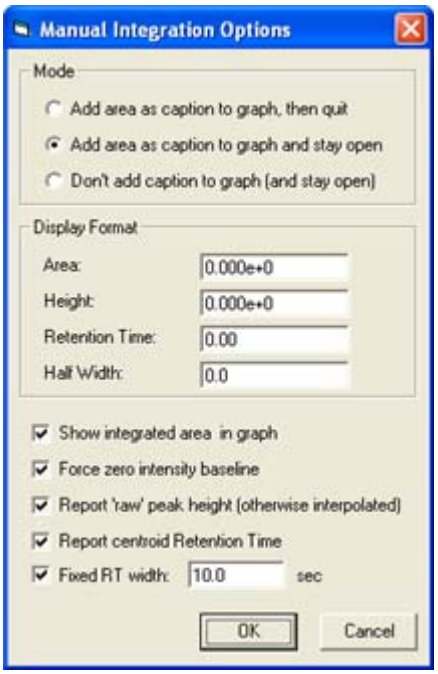


Figure 1-20 Manual Integration Options

7. If required, in the **Mode** group, do one of the following:
   - Click **Add area as caption to graph, then quit** to paste the area of a single selection on the graph and then exit the program.
   - Click **Add area as caption to graph and stay open** to display the area on the graph as well as in the program.
   - Click **Don't add caption to graph (and stay open)** to only display the results in the program and to leave the graph unaltered.
8. Select the following as required:
   - In the **Display Format** group, select how the results will be displayed in the Manual Integration 1 dialog.
   - Select the **Show integrated area in graph** check box to display the integrated area in the active chromatogram.
   - Select the **Force zero intensity baseline** check box to force the integrated area to start from the intensity=0 baseline. In this case, the starting and ending times from the manual selection are used, but the y-positions are ignored.
   - Select the **Report 'raw' peak height** check box to report the peak height as the intensity of the largest point comprising the peak. If the check box is cleared, then the usual software algorithm is used to calculate the peak height (a parabola is fitted to the three largest data points and the peak height is set to the y-value of the parabola's apex).
   - Select the **Report centroid Retention Time** check box to report the retention time using a centroid calculation. If the check box is cleared, then the usual software algorithm is used to calculate the retention time (a parabola is fitted to the three largest data points and the retention time is set to the x-value of the parabola's apex).

- The **Fixed RT width: _ sec** option is for MALDI workflows only. If selected, the total width of the resulting peak is fixed at the specified value and is centered at the apex retention time.

9. To save changes and return to the **Manual Integration 1** dialog, click **OK**.
10. On the **Manual Integration 1** dialog, click **Close**

Mascot

Use this script to send either the active spectrum or all product spectra contained in the active sample, or all samples in the active data file, to the Mascot protein search engine. The script was co-developed with Matrix Science Limited, the creators of Mascot.

When sending only the active spectrum, the script can work with either MS or MS/MS data; in the first case a peptide mass fingerprint search is conducted. When sending all spectra, the script works with data acquired using two distinct types of acquisition methods: either a multiple period / multiple experiment method containing any number of product experiments or an IDA (Information Dependent Acquisition) method. In the former case, the script calculates one spectrum for each experiment by averaging all spectra acquired for the experiment. In the latter case, the script uses all dependent product spectra, combining adjacent spectra with the same parent mass and charge state.

To use the script

If you are planning to send just one spectrum to Mascot, make sure that a centroided spectrum is active in the Analyst® software before running the script. If you are planning to send all spectra contained in a sample, the TIC for the sample should be active.

1. With a centroided spectrum in an active pane, click **Script** > **Mascot**.

   The File, Sample, Periods, and Scan type IDA data fields are read-only and show information about the active sample.

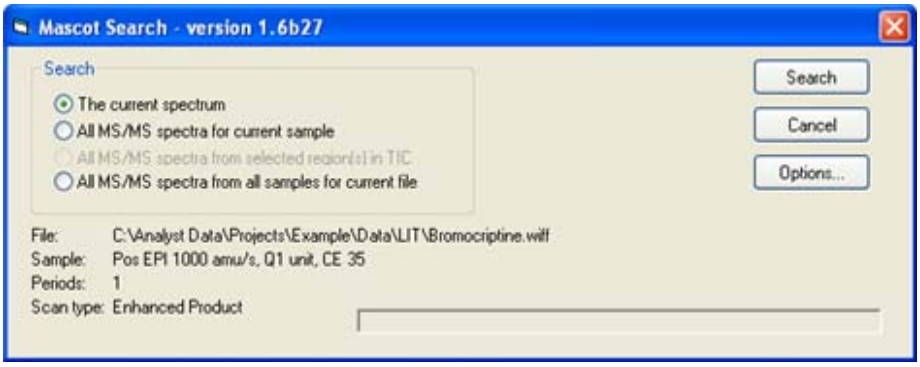

Mascot Search - version 1.6b27
Search
The current spectrum
All MS/MS spectra for current sample
All MS/MS spectra from selected region(s) in TIC
All MS/MS spectra from all samples for current file
Search
Cancel
Options...
File: C:\Analyst Data\Projects\Example\Data\LIT\Bromocriptine.wiff
Sample: Pos EPI 1000 amu/s, Q1 unit, CE 35
Periods: 1
Scan type: Enhanced Product

Figure 1-21 Mascot Search dialog

2. In the **Search** group, you can do one of the following:
   - If the active pane is a centroided spectrum (either MS or MS/MS), click **The current spectrum** to search this spectrum on its own. You can select this option only if the spectrum was centroided using the **Centroid** command on the **Explore** menu.
   - If the active pane contains MS/MS data, click **All MS-MS spectra for current sample** to perform a single search using all spectra for the sample.
   - If the active pane contains MS/MS data and also contains a selected region, click **All MS-MS spectra from selected region in TIC** to perform a single search using only the spectra from this region. This can be particularly useful to speed the generation of the search input if only a portion of the run is known to contain useful data.

- If the active pane contains MS/MS data and the associated data file contains more than one sample, click **All MS-MS spectra from all samples for current file** to perform a single search using all MS/MS spectra from all samples in the data file.

3. To open the Mascot search form and populate it with the appropriate information, click **Search**. If you are searching all product spectra contained in the sample, this may take some time (a progress bar will appear). After the Web form appears, click **Start Search**.
4. To set the various search options, click **Options**.

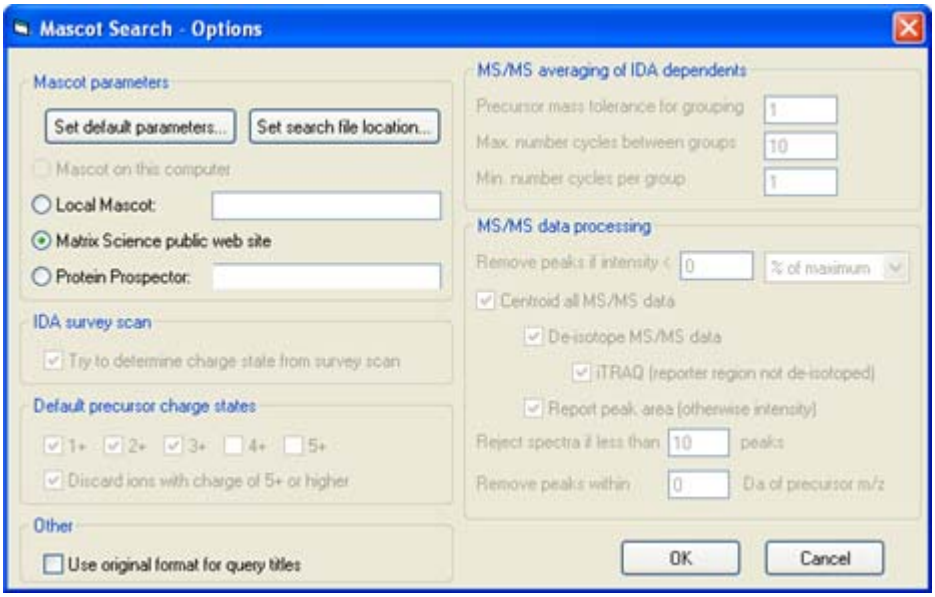


Figure 1-22 Mascot Search-Options dialog

Tip! To display the Mascot search form defaults Web page, click **Set default parameters**.

5. To set where the text file used as input to Mascot is located, click **Set search file location**, select one of the following options and then click **OK**.

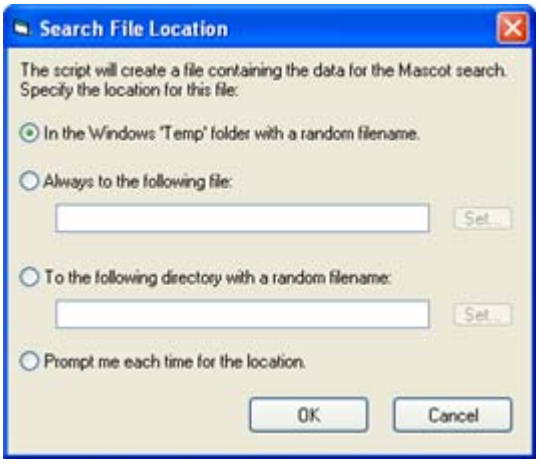

Search File Location
The script will create a file containing the data for the Mascot search. Specify the location for this file:
In the Windows 'Temp' folder with a random filename.
Always to the following file:
Set...
To the following directory with a random filename:
Set...
Prompt me each time for the location.
OK
Cancel

Figure 1-23 Search File Location dialog

- To place the file in the Windows temporary folder with a random but unique file name, click **In the Windows 'Temp' folder with a random filename**.
- To always write to a specific file, overwriting the file for every search, click **Always to the following file**. To navigate to the folder, click **Set**.
- To create the file with a random but unique filename in the specified directory, click **To the following directory with a random filename**. To navigate to the folder, click **Set**.
- To be prompted to save every time a search is performed, click **Prompt me each time for the location**.

6. In the **Default precursor charge states** group when searching only the current spectrum, values here are not available; the precursor charge state should be set manually using the resulting Mascot web form. When searching product spectra for an IDA run, the charge state is automatically determined by the script—the values specified here are not used unless the charge state could not be automatically determined or the **Try to determine charge state from survey scan** check box is not selected. When searching product spectra for a multiple period or multiple experiment LC/MS run, the specified charge states will always be considered for each product spectrum.

   This option is only required for those using an older Mascot software version that does not accept charge states greater than 5. If you are using one of these older versions, make sure that in the Default precursor charge states group, the Discard ions with charge of 5+ or higher check box is selected. Some versions of the Mascot search engine cannot accept ions with a charge state of 5 or higher and show a warning for each ion exceeding this limit.

7. To use the default precursor charge states as is, clear the **Try to determine charge state from survey scan** check box. Otherwise, it will attempt to determine the charge state by examining the isotope spacing in the survey spectrum, and for Analyst TF 1.5 software data, it will use the charge state determined by the MS Acquisition Engine, which is saved to the data file. If this check box is selected but the charge state determination fails, the default charge states are used.

8. In the **MS/MS averaging of IDA dependents** group, edit the parameters that pertain to the calculation of the product ion spectra for an IDA run.

- The **Precursor mass tolerance for grouping** field is used to potentially combine adjacent product spectra into a single spectrum. If two spectra have precursors with the same charge state and the same MW within this tolerance, they will be combined.
- In the **Max. number cycles between groups** field, spectra are not combined if the number of cycles between spectra with the same *m/z* and charge state is greater than the specified value. Use this option if you do not want to combine spectra with significantly different retention times.
- In the **Min. num cycles per group** field, type the minimum number of spectra that need to be combined in order for the result to be kept.

Note: If you have used the dynamic exclusion IDA option, set this value to 1.

9. In the **MS/MS data processing** group, select parameters that pertain to the filtering of product ion spectra.
   - **Remove peaks if intensity <** _ — Removes peaks that are either less than a specific count or a specific percent of the maximum peak intensity of the spectrum.
   - To centroid the MS/MS spectra before sending them to Mascot for searching, select the **Centroid all MS/MS data** check box. It is highly recommended that you enable the centroid option.
   - If the centroid option is used, indicate whether isotope peaks should be removed from the MS/MS spectra before sending them to Mascot by selecting the **De-isotope MS/MS data** check box. It is recommended that you enable this option.
   - **Report peak area (otherwise intensity)**—If selected, the script uses the area of the peak. Otherwise it uses the intensity at the apex.
   - **Reject spectra if less than 'n' peaks** —If a spectrum contains unreasonably few peaks after combining adjacent spectra (if used) and centroiding (if used), the spectrum can be eliminated.
   - **Remove peaks within 'n' Da of precursor m/z**— Sets a window around the precursor ion *m/z* and then removes any peaks within that window.
10. In the **Other** group, select **Use original format for query titles** if you are using a third-party protein quantitation application and you would like to use the original title format.
11. To display the Mascot search form defaults Web page, click **Set default parameters**. You can edit the various defaults so that you do not need to reset them manually every time before submitting a search. After changing the parameters, click **Save defaults as cookie** to close the Web page.

Mass Defect Filter

The identification of drug metabolites in biological fluids with low concentrations from a total ion chromatogram (TIC) is challenging because of significant interferences from endogenous species. A technique of filtering the data based on the mass defect of the parent drug and a small tolerance value has been used to decrease the amount of interference. Using this technique aids in the identification of phase I and phase II metabolites.

The Mass Defect Filter script filters either a TIC or a spectrum using this technique. Only those data points in the spectrum whose centroid mass is within the tolerance range of the parent ion's mass defect applied at that nominal mass will be retained. All other points are excluded. Summing the intensity for each of the spectra generates the TIC. A further filtering based on absolute mass can also be applied.

A new graph of the filtered TIC or spectrum will appear below the current graph.

To use the script

1. With either a spectrum or a TIC active, click **Script** > **Mass Defect Filter**.

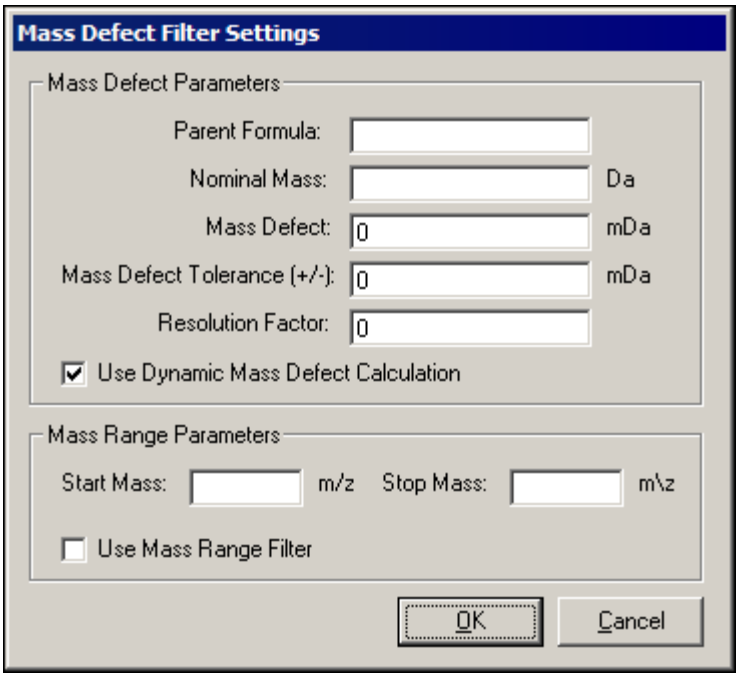


Figure 1-24 Mass Defect Filter Settings dialog

2. In the **Parent Formula** field, type the formula for the parent ion. The **Mass Defect** and **Nominal Mass** fields are automatically updated.
3. If the parent formula is unknown, then type values in the **Nominal Mass** and **Mass Defect** fields.
4. In the **Mass Defect Tolerance (+-)** field, type the tolerance value.
5. If required, type a value in the **Resolution Factor** field. The Resolution Factor further filters the data by keeping only the centroid values whose resolution is greater than or equal to it.

6. If required, to allow the mass defect to be applied differently at each nominal mass in the spectrum, click the **Use Dynamic Mass Defect Calculation** check box. If you deselect the check box, a constant value of the mass defect is added to each nominal mass.
7. In the **Mass Range Parameters** group, select the **Use Mass Range Filter** check box to set the mass range parameters. Only masses in the spectrum between **Start Mass** and **Stop Mass** inclusively will be retained.
8. Click **OK** to start processing.

Merge MRM

Use this script to add all experiment data from the method to be merged to the base method. Both methods should have only one period and one experiment. The script does not limit the number of mass ranges in an experiment. All mass ranges are saved to the base method.

This script can be used to merge multiple final methods created by Compound Optimization. After you have merged the methods, the LC information can be modified if necessary to reflect the analysis conditions.

To use the script

1. Click **Script** > **Merge MRM**.

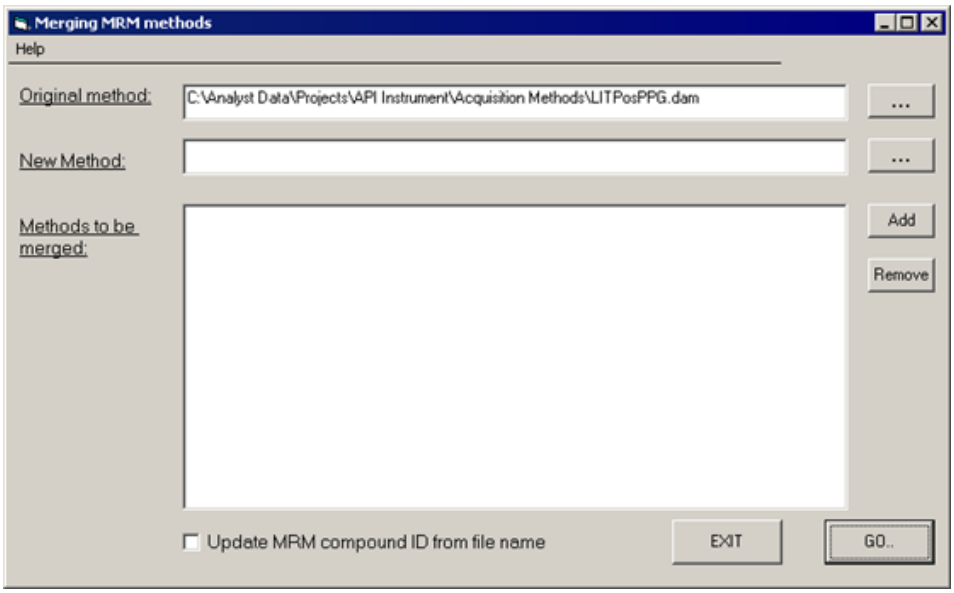


Figure 1-25 Merging MRM methods dialog

2. To navigate to the original acquisition method file or template, click the button to the right of **Original method**.
3. To specify the name and location of the merged acquisition method file, click the button to the right of **New Method**, type the name of the method, and then click **Open**.
4. To add an acquisition method to the list of methods to be merged, click **Add** to the right of **Methods to be merged**. To remove a method from the list, click **Remove**.
5. (Optional) If both methods were created using Compound Optimization, select the **Update MRM compound ID from the file name** check box to populate the compound ID column with the compound name in the merged method.
6. To add all the mass ranges from the selected methods, click **Go**.

MRM3 Optimization Script

Use this script for quantitation on the QTRAP® systems in order to provide increased specificity and, therefore, improved detection when quantifying analytes in complex matrices. This script is designed to generate an optimal MS^3 acquisition method on any QTRAP system with any source and at any flow using infusion. The script performs the following optimization steps:

- Confirm precursor mass
- Optimize transmission to collision cell
- Determine the major fragment ions
- Optimize the Collision Energy (CE) for each fragment ions
- Perform MS^3 scans on each fragment ion
- Optimize Excitation Energy (AF2) for all MS^3 scans
- Generate a report
- Save all data and acquisition methods

The script can also be used in qualitative applications to generate collections of MS/MS and MS3 spectra for compounds in a semi-automated way (that is, one compound at a time).

MRM3 Optimization Window Overview

You can use the controls in the MRM3 Optimization window to navigate through the script. The window also displays the optimization results as they are generated. The following is an overview of the various sections in this window.

- **Status Window**: When the script is first started, this window displays the current optimization settings that will be used for optimization. When the optimization is started, spectral information is displayed in this window.
- **Log File**: Displays the results found during optimization in text format. Each entry found in this section is also added to the generated Log.txt file.
- **Overall Progress**: This is a visual display of the overall optimization progress.
- **Main Controls**: Contains all of the main functions associated with the setting and execution of the optimization process.
    - After the optimization is completed, a Results.txt file is automatically generated and saved. If you click View Results, you can open and review it with Microsoft Notepad.
    - Click **Settings** to open a window where you can type compound information required for the optimization process.
    - Click **Start** to initiate the optimization process. During optimization, this button is renamed to Abort, which you can click to stop the optimization process.

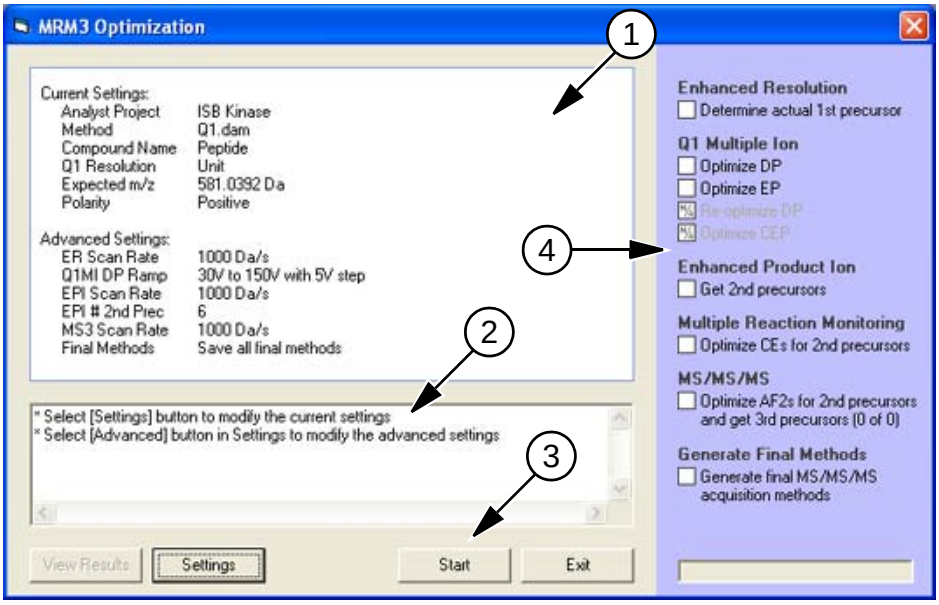


Figure 1-26 MRM3 Optimization window

Item	Description
1	Status Window
2	Log File
3	Main Controls
4	Overall Progress

To set the preferences

When the script is run for the first time, the Settings dialog appears automatically. Otherwise, the MRM3 Optimization window appears, displaying the last values and information used for optimization.

1. Click **Browse** to navigate to the starter acquisition method. This method predominantly contains the source conditions to be used for the optimization.
2. In the **Compound Name** field, type a descriptive compound name. This name is used as a prefix to all of the acquisition methods and data files generated.
3. In the **Q1 Resolution** field, select a Q1 Resolution to be used for MS/MS and MS3.
4. In the **Polarity** group, click a polarity, which may differ from the starter method. The Both Polarity option is currently not supported.
5. In the **Expected m/z (amu)** field, type the expected mass-to-charge (*m/z*) ratio for the compound. If you do not know the *m/z* of the compound, then click **Calculate from chemical formula** to calculate it from the chemical formula of the compound. See To calculate m/z on page 57.
6. To modify some of the settings used by the optimization process, click **Advanced**. See To use the Advanced Settings dialog on page 58.
7. To verify and use the updated settings, click **OK**.

To use the script

1. Build a starter acquisition method if one does not already exist. The starter method should be a Q1 acquisition method created in Manual Tune and should contain the source conditions required for the tuning process because these are not optimized by the script.
2. Save the method in the Acquisition Methods folder of the required project where all generated files will be saved.
3. Click **Script** > **MRM3 Optimization**.

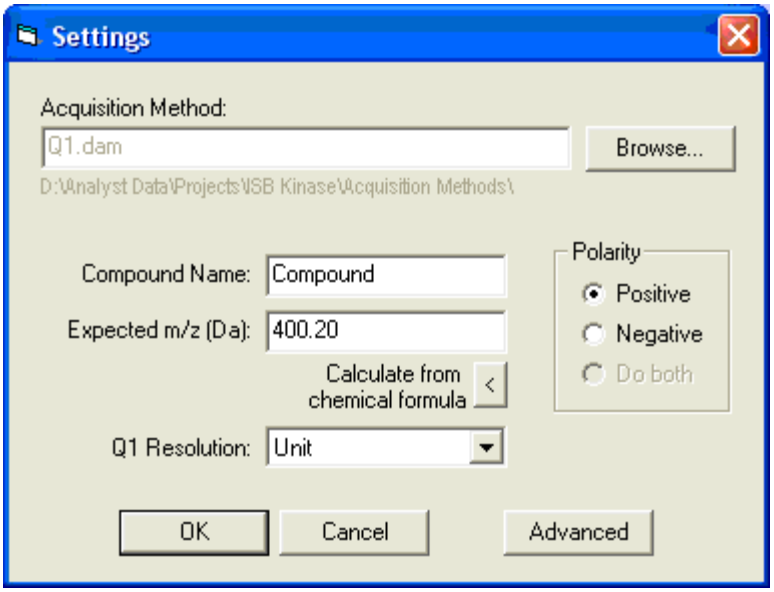


Figure 1-27 Settings dialog

4. Enter the compound information required for the optimization process and then click **OK** on the **Settings** dialog.
5. To initiate the optimization process, click **Start** in the **MRM3 Optimization** window.

To calculate *m/z*

The *m/z* calculator is accessed through the Settings dialog.

1. In the **MRM3 Optimization** window, click **Settings**.
2. In the **Settings** dialog, click **Calculate from chemical formula**.

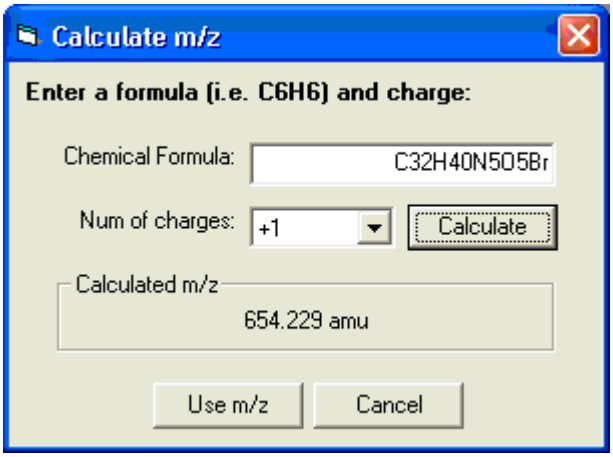


Figure 1-28 Calculate m/z dialog

3. In the **Chemical Formula** field, type the chemical formula of the compound. Use capital letters for elements. The chemical formula for peptides is also entered into this dialog. You can obtain the chemical formulas for peptides by typing the peptide sequence into the New Protein Sequence window in the BioAnalyst™ Software.
4. In the **Num of charges** field, click the number of charges.
5. To calculate the *m/z* for the entered chemical formula and charge, click **Calculate**.
6. To close the calculator and update the **Expected m/z (amu)** field in the **Settings** dialog with the calculated *m/z*, click **Use m/z**.

To use the Advanced Settings dialog

In this dialog, a description for each of the optimization steps is provided. You can also modify some of the settings in order to customize the optimization.

1. In the **MRM3 Optimization** window, click **Settings**.
2. In the **Settings** dialog, click **Advanced**.

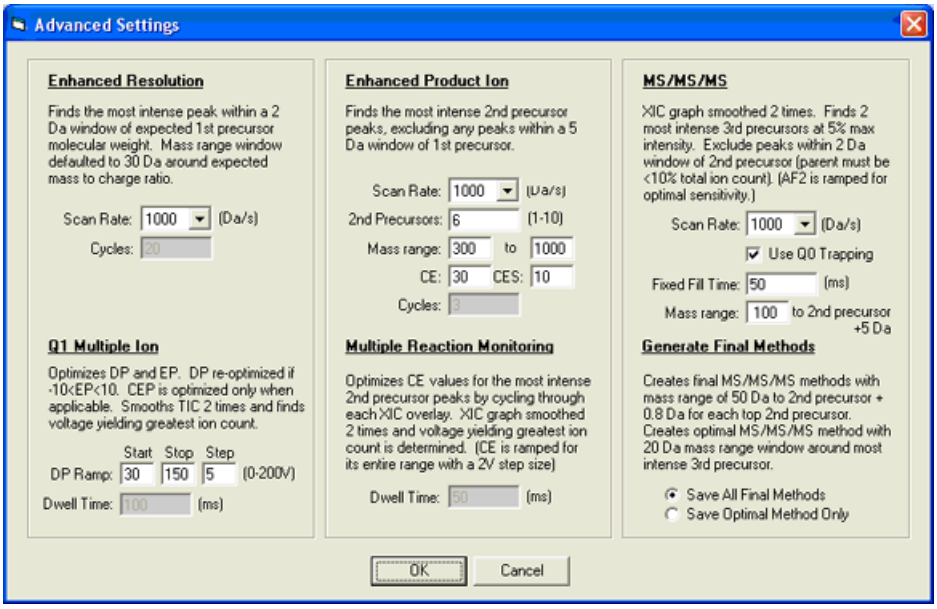

Advanced Settings
Enhanced Resolution
Finds the most intense peak within a 2 Da window of expected 1st precursor molecular weight. Mass range window defaulted to 30 Da around expected mass to charge ratio.
Scan Rate: 1000 (Da/s)
Cycles: 20
Enhanced Product Ion
Finds the most intense 2nd precursor peaks, excluding any peaks within a 5 Da window of 1st precursor.
Scan Rate: 1000 (Da/s)
2nd Precursors: 6 (1-10)
Mass range: 300 to 1000
CE: 30 CES: 10
Cycles: 3
MS/MS/MS
XIC graph smoothed 2 times. Finds 2 most intense 3rd precursors at 5% max intensity. Exclude peaks within 2 Da window of 2nd precursor (parent must be <10% total ion count). (AF2 is ramped for optimal sensitivity.)
Scan Rate: 1000 (Da/s)
Use Q0 Trapping
Fixed Fill Time: 50 (ms)
Mass range: 100 to 2nd precursor +5 Da
Q1 Multiple Ion
Optimizes DP and EP. DP re-optimized if -10<EP<10. CEP is optimized only when applicable. Smooths TIC 2 times and finds voltage yielding greatest ion count.
Start Stop Step
DP Ramp: 30 150 5 (0-200V)
Dwell Time: 100 (ms)
Multiple Reaction Monitoring
Optimizes CE values for the most intense 2nd precursor peaks by cycling through each XIC overlay. XIC graph smoothed 2 times and voltage yielding greatest ion count is determined. (CE is ramped for its entire range with a 2V step size)
Dwell Time: 50 (ms)
Generate Final Methods
Creates final MS/MS/MS methods with mass range of 50 Da to 2nd precursor + 0.8 Da for each top 2nd precursor. Creates optimal MS/MS/MS method with 20 Da mass range window around most intense 3rd precursor.
Save All Final Methods
Save Optimal Method Only
OK
Cancel

Figure 1-29 Advanced Settings dialog

3. In the **Scan Rate** fields in the Enhanced Resolution, Enhanced product Ion and MS/MS/MS groups, select a scan rate for **ER**, **EPI**, and **MS**^{3}.
4. In the **Q1 Multiple Ion** group, in the **DP Ramp** fields, type the declustering potential (DP) range for optimization. The range is expressed in absolute values and the appropriate polarity is automatically applied based on the selection made in the Settings dialog.
5. In the **Enhanced Product Ion** group, do the following:
   - In the **2nd Precursors** field, type the maximum number of second precursors (fragment ions) used for MS^3 optimization. Type a number between 1 and 10.
   - In the **Enhanced Product Ion** group, in the **Mass range** field, type a mass range for the second precursors that will be selected for MS^3 optimization.
   - In the **CE** field, type a collision energy value and in the **CES** field, type a collision energy spread (CES) value that will provide a good MS/MS spectrum from which fragment ions can be selected.
6. To generate all of the final MS^3 methods for each second precursor and the optimal MS3 method for quantitation, in the **Generate Final Methods** group, click **Save All Final Methods**. Click **Save Optimal Method Only** to save only the optimal MS^3 method (most sensitive for quantitation).
7. Click **OK** to accept the updated Advanced Settings.

Optimization In Progress

When the optimization is started, Manual Tune in the Analyst® software is automatically stopped. While the script is running, all of the functions in the software can still be used. A Log.txt file is also updated as each part of the optimization procedure is completed. To stop the script at any time, click the Abort button. Examples of the script in progress are shown in the <Emphasis>MRM3 Optimization window after EPI scan figure on page 60 and the

<Emphasis>MRM3 Optimization window during MS3 scan figure on page 61. In the Overall Progress section, the Checklist images and text fonts represent different statuses that are described in the following section.

- ☐ Task not performed yet – text is black
  Task in progress – text is blue and italic
- ☒ Task will not be performed – text is grey
- ☑ Task completed (hyperlink) – text is blue and underlined
  Task completed (no link) – text is blue
  Part of task completed (hyperlink) – text is blue, underlined, and italic

When the text is underlined, you can click it like a web page hyperlink and the corresponding spectrum or chromatogram is displayed. The text found under MS/MS/MS also displays the MS^3 scan number that is being performed because it is possible to have between 1 and 10 scans. The Overall Progress section also includes a Message area. In this area, a progress bar displays the current step progress. Above the progress bar, various messages are displayed such as the time and other statuses for the current optimization step.

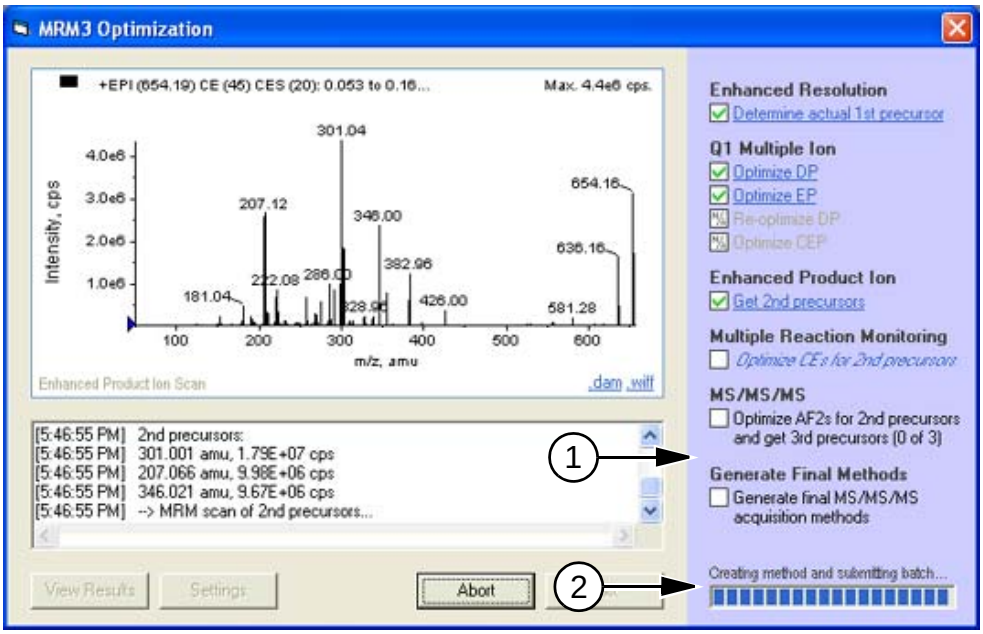


Figure 1-30 MRM3 Optimization window after EPI scan

Item	Description
1	Checklist
2	Message

In the spectral status window, the previously generated spectrum or chromatogram is displayed. When one of the checklist items is selected, the corresponding graph is displayed. The scan type name indicates which scan is currently being displayed. For each completed step, it is possible to open the acquisition method (.dam) or data file (.wiff) associated with the graph displayed. If an MS/MS/MS scan is displayed, you can use the buttons to cycle through the different MS^3 scans.

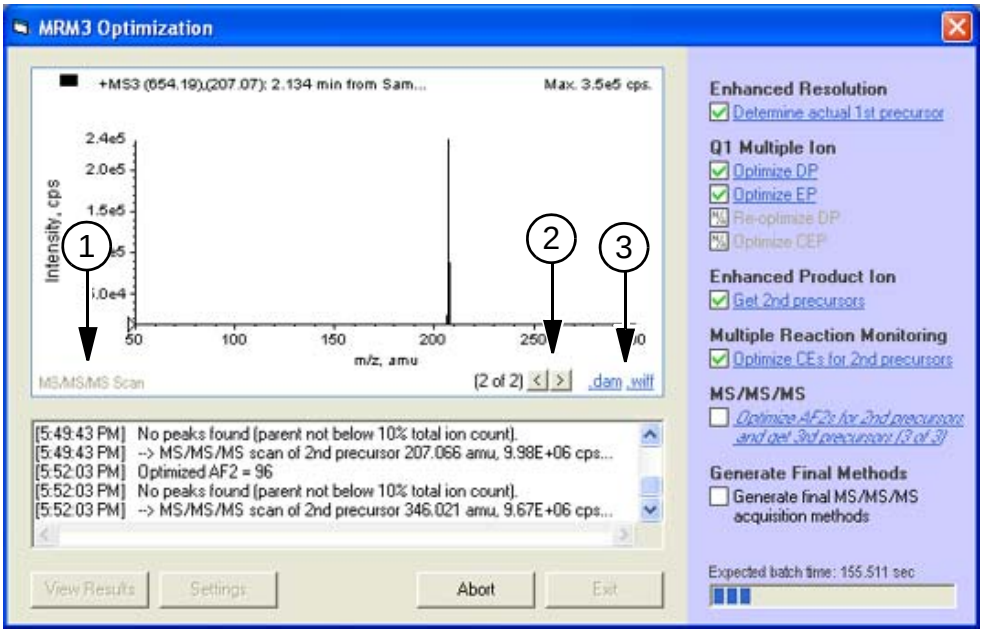

MRM3 Optimization
+MS3 (654.19)(207.07): 2.134 min from Sam...
Max. 3.5e5 cps.
Intensity, cps
m/z, amu
MS/MS/MS Scan
(2 of 2)
.dam .wiff
[5:49:43 PM] No peaks found (parent not below 10% total ion count).
[5:49:43 PM] --> MS/MS/MS scan of 2nd precursor 207.066 amu, 9.98E+06 cps...
[5:52:03 PM] Optimized AF2 = 96
[5:52:03 PM] No peaks found (parent not below 10% total ion count).
[5:52:03 PM] --> MS/MS/MS scan of 2nd precursor 346.021 amu, 9.67E+06 cps...
View Results
Settings
Abort
Exit
Enhanced Resolution
Determine actual 1st precursor
Q1 Multiple Ion
Optimize DP
Optimize EP
Re-optimize DP
Optimize CEP
Enhanced Product Ion
Get 2nd precursors
Multiple Reaction Monitoring
Optimize CEs for 2nd precursors
MS/MS/MS
Optimize AF2s for 2nd precursors and get 3rd precursors (3 of 3)
Generate Final Methods
Generate final MS/MS/MS acquisition methods
Expected batch time: 155.511 sec

Figure 1-31 MRM3 Optimization window during MS^3 scan

Item	Description
1	Scan type
2	Buttons to cycle through different MS^3 scans
3	Links

Optimization Complete

When the quantitative optimization for MS^3 is completed or stopped, a Results.txt file is generated. This file is automatically opened in Microsoft Notepad. You can also view the file by clicking View Results from the MRM3 Optimization window. The various parts of the Results.txt file are described as follows.

- **Time and Duration:** Displays the date and time duration of optimization.
- **User Starting Conditions**: Displays the settings and Advanced Settings in this section.
- **Optimization Conditions Found**: Displays the optimal conditions found during the ER and Q1MI scans.
- **MS3 Fragments Found and Associated Losses**: Displays the fragments and optimal conditions (collision energy and excitation energy) as well as associated losses found for the EPI scan and MS^3.

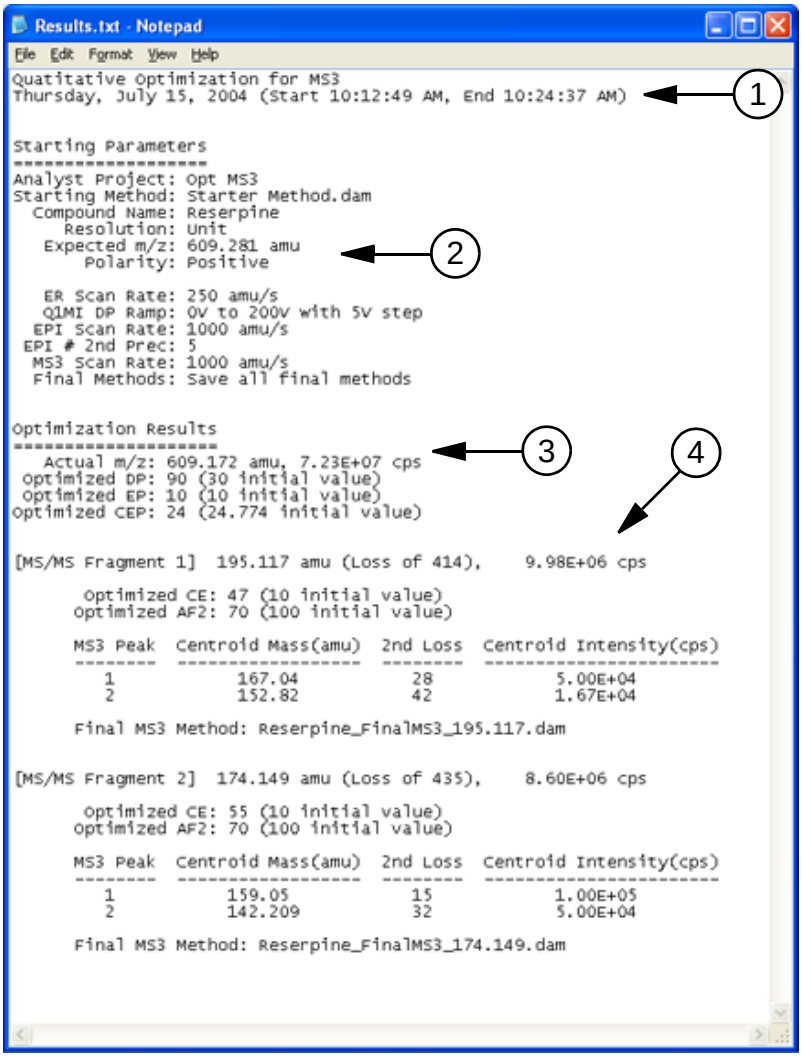

Results.txt - Notepad
File Edit Format View Help
Quatitative Optimization for MS3
Thursday, July 15, 2004 (Start 10:12:49 AM, End 10:24:37 AM)
Starting Parameters
Analyst Project: Opt MS3
Starting Method: Starter Method.dam
Compound Name: Reserpine
Resolution: Unit
Expected m/z: 609.281 amu
Polarity: Positive
ER Scan Rate: 250 amu/s
Q1MI DP Ramp: 0V to 200V with 5V step
EPI Scan Rate: 1000 amu/s
EPI # 2nd Prec: 5
MS3 Scan Rate: 1000 amu/s
Final Methods: Save all final methods
Optimization Results
Actual m/z: 609.172 amu, 7.23E+07 cps
Optimized DP: 90 (30 initial value)
Optimized EP: 10 (10 initial value)
Optimized CEP: 24 (24.774 initial value)
[MS/MS Fragment 1] 195.117 amu (Loss of 414), 9.98E+06 cps
Optimized CE: 47 (10 initial value)
Optimized AF2: 70 (100 initial value)
MS3 Peak Centroid Mass(amu) 2nd Loss Centroid Intensity(cps)
1 167.04 28 5.00E+04
2 152.82 42 1.67E+04
Final MS3 Method: Reserpine_FinalMS3_195.117.dam
[MS/MS Fragment 2] 174.149 amu (Loss of 435), 8.60E+06 cps
Optimized CE: 55 (10 initial value)
Optimized AF2: 70 (100 initial value)
MS3 Peak Centroid Mass(amu) 2nd Loss Centroid Intensity(cps)
1 159.05 15 1.00E+05
2 142.209 32 5.00E+04
Final MS3 Method: Reserpine_FinalMS3_174.149.dam
1
2
3
4

Figure 1-32 Optimization Report

Item	Description
1	Time and Duration
2	User Starting Conditions
3	Optimization Conditions Found
4	MS3 Fragments Found and Associated Losses

All of the generated acquisition methods have a descriptive file name in the format [supplied compound name] + [scan type] + [*m/z*] + .dam. These methods are saved in the same folder as the starter acquisition method.

All of the data, Log.txt, and Results.txt files are saved into a Data sub-folder that is created in the same project as the starter acquisition method. The sub-folder has the format [supplied compound name] + OptMS3 + ([date], [time]). The data files have the format [supplied compound name] + [scan type] + [*m/z*] + .wiff.

Detailed Description of Script Logic

This section describes each phase of the optimization process. All scans are performed with the number of scans to sum set to 3.

Initialization

Before performing any optimization scans, the MRM3 Optimization script first performs the following initialization steps. If an error occurs during any of these steps, the script will stop the optimization process.

1. Ensure that the Analyst software is running.
2. Load the starter acquisition method to see if it is valid and check the device type.
3. Create a new Data sub-folder to store the .wiff files.
4. Create the Log.txt file.

Enhanced Resolution Scan

This step confirms the mass of the ion used for optimization. The ER scan is performed for 20 cycles at the specified scan rate. The most intense peak within ±1 amu of the expected first precursor *m/z* is then selected. Similar to the Analyst software, this scan is performed with a 30 amu mass range around the specified *m/z*. For multiply charged species, the C12 ion is determined in this step.

Q1 Multiple Ion Scan

This step optimizes transmission of the ion of interest up to the collision cell. This is performed using a Q1MI scan. The script first optimizes the DP parameter by performing the scan at the specified DP ramp. Optimize the EP parameter by ramping it from 1 V to 12 V (-12 V to -1 V for negative mode), with 0.5 V step. If the optimal EP is less than 10 V (greater than -10 V for negative mode), then DP is re-optimized. If the instrument is not a 4000 QTRAP instrument, the CEP parameter is also optimized by ramping from 0 to 100 V (-100 to 0 V for negative mode) with 2 V step. In determining the optimal voltage, graphs are smoothed two times and the voltage yielding the greatest ion count is used. Dwell Time for each scan is set to 100 ms.

Enhanced Product Ion Scan

This step selects the fragment ions that will be used for MS^3 optimization. This is performed using an EPI scan for three cycles at the selected scan rate. You can specify an optimal CE for the compound to be analyzed. If the optimal CE is unknown, then you can specify a Collision Energy Spread (CES) value such that a range of CE settings are used. The most intense second precursor peaks are then found, excluding any peaks within ±2.5 amu window of first precursor. The number of second precursors to use is selected in the Advanced Settings. The mass range from which the second precursors are selected is specified by the user.

Multiple Reaction Monitoring Scan

This step optimizes the collision energy for each of the fragment ions selected from the EPI scan. This is performed using MRM scans. Use CE ramps of 5 to 130 V (-130 to -5 V in negative mode)

with 2 V step and Dwell Time of 50 ms. Each overlaid graph is then smoothed two times and the voltages yielding the greatest ion count are used as the optimal CE values.

MS/MS/MS Scan

The script performs an MS^3 scan for each chosen second precursor at the specified scan rate and with an AF2 ramp of 0 to 100 V with 2 mV step (or 0 to 0.4 V with 0.01 V step on QTRAP® 5500 system) for both polarities. The fill time of the scan is set, and Q0Trapping can be turned on for maximum sensitivity if required. The lower limit of the mass range for the MS/MS/MS scan can be specified, and the upper limit is second precursor + 5 amu.

The generated graphs are smoothed twice and the optimal AF2, as shown in the the following figure, <Emphasis>How AF2 is determined, is obtained when the residual intensity of the second precursor (based on XIC) is at 5% of its maximum intensity. The spectrum at this AF2 value is then used to find the two most intense second generation fragment ions, excluding peaks within ±1 amu of the second precursor. If the second precursor *m/z* is greater than 10% of the total ion count, no fragments from that spectrum will be used. This condition exists because if the second precursor *m/z* is greater than 10%, there is insufficient fragmentation.

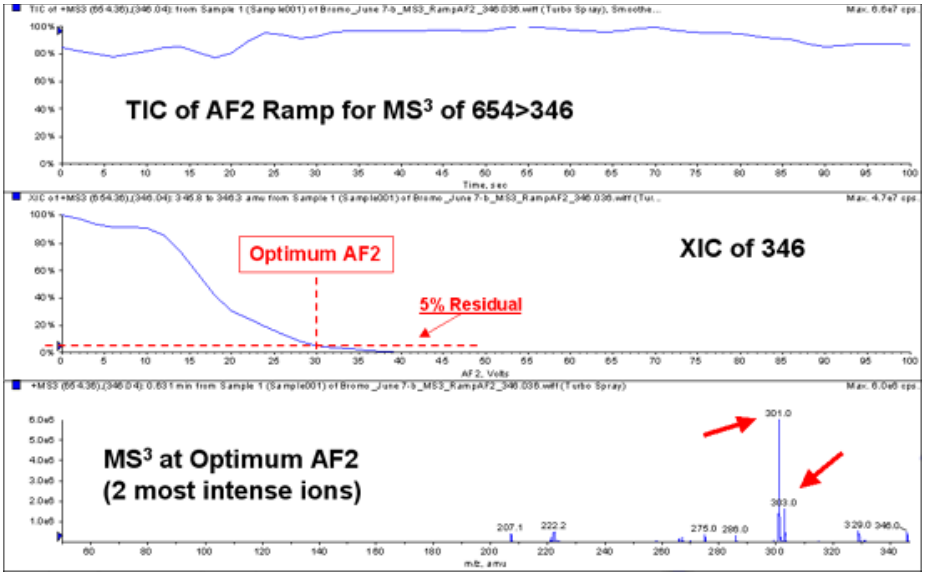


Figure 1-33 How AF2 is determined

Generate Final Methods

After the optimization scans are performed, the script generates the final MS^3 methods. If the Save Optimal Method Only option is clicked in the Advanced Settings dialog, only an optimal MS3 method with ±10 amu around the most intense second generation fragment ion is created. If the Save All Final Methods option is clicked, then the optimal method as well as an MS3 method for each of the top second precursors are created using a mass range from the user-defined lower limit to an upper limit of (second precursor + 5) amu.

Multiple Batch Scripts

Use this script to attach multiple acquisition scripts to a single batch that is submitted to the queue. These acquisition scripts are used to immediately process the data either after a sample completes or after the batch finishes. Using the Analyst® software, you can submit only one script with the batch, but sometimes it is convenient to run two or more scripts to perform two or more different types of processing.

To use the script

1. When creating a batch, in the **Batch Script** field, navigate to the script.

   After submitting the batch to the queue, the following dialog appears in which you can attach the additional acquisition scripts.

Figure 1-34 Multiple Batch Scripts Script dialog

2. To attach an additional script to this batch, click **Add Script** to navigate to the acquisition script.
3. To remove a script, click the script and then click **Remove Selected**.
4. Select the **Only show this dialog again if the control key is down** check box if you want the dialog to appear when submitting the batch.
5. Click **Run** to attach all of the acquisition scripts to the batch.

Open In Workspace

Use this script to open a sample in a previously created workspace. This script loads the previous workspace and displays the sample in the same layout as specified in the saved workspace. It is not possible to create a workspace that specifies pane arrangements so that any sample can be loaded into it. For more information on workspaces, refer to the online Help.

To use the script

1. Click **Script** > **OpeninWorkspace**.

   The Open in Workspace dialog appears with the current working project data folder loaded into the Data Files list.

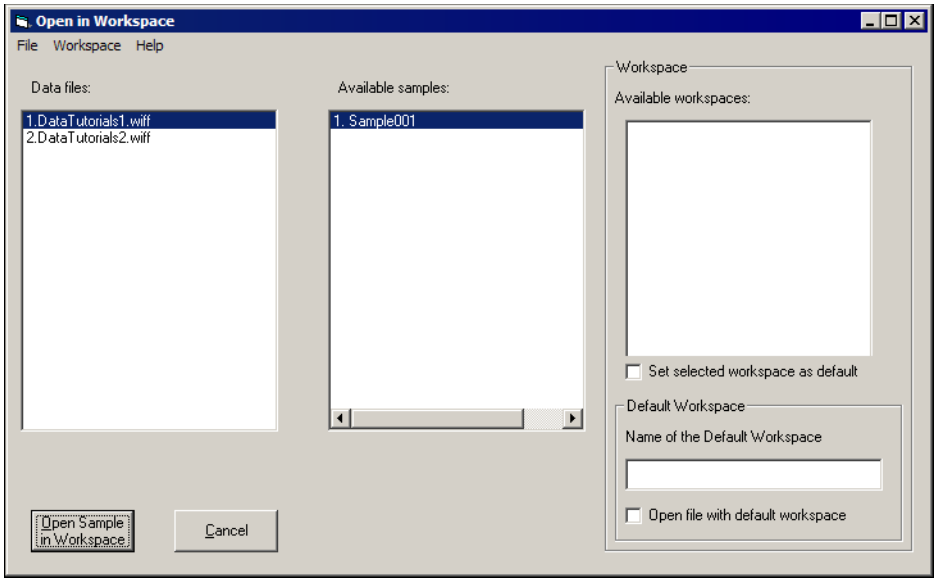


Figure 1-35 Open in Workspace dialog

2. To select a data file from another project, click **File** > **Open Data File**.
3. In the **Data files** list, click a data file.

   All the samples in the selected data file appear in the Available Samples list
4. After choosing the sample, select a workspace in the **Available workspaces** list. To select a different workspace, click **Workspace** > **Open Workspace**.
5. To set a default workspace to be used each time this script is run, select the **Set Selected Workspace As Default** check box.
6. To open the sample, click **Open Sample in Workspace**.

Known Issues and Limitations

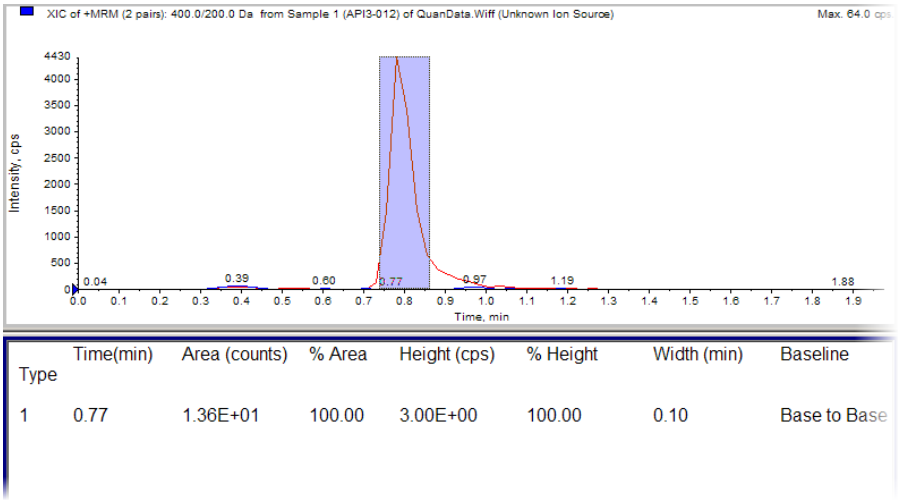
The Open in Workspace script is for the presentation of graphs only. The script cannot handle data lists that are saved in the workspace and a Type Mismatch error will be displayed. You must save your workspaces with the graphs only first and then, after loading the workspace, create the necessary data lists.

Peak List from Selection

Use this script to determine the peak data for a selected region or regions in the chromatogram. The %area and %height listed relate just to peaks in the selection. The peaks are listed in a text pane below the active chromatogram. The peak definition is shown in the chromatogram. For more information, see the following figure, <Emphasis>Data Processing with the PeakListFromSelection Script.

To use the script

- Do one of the following:
  - To run the script, click **Script** > **PeakListFromSelection**.
  - To see the script description, and get the peak list, hold the Shift key down while clicking the script.
  - To process the data, make one or more selections in the chromatogram and then select the script from the drop-down menu.



Type	Time(min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline
1	0.77	1.36E+01	100.00	3.00E+00	100.00	0.10	Base to Base

Figure 1-36 Data Processing with the PeakListFromSelection Script

Regression Calculator

Use this script to calculate the slope, y intercept, and r values for each mass/speed dependent parameter.

To use the script

1. Click **Script** > **Regression Calculator**.

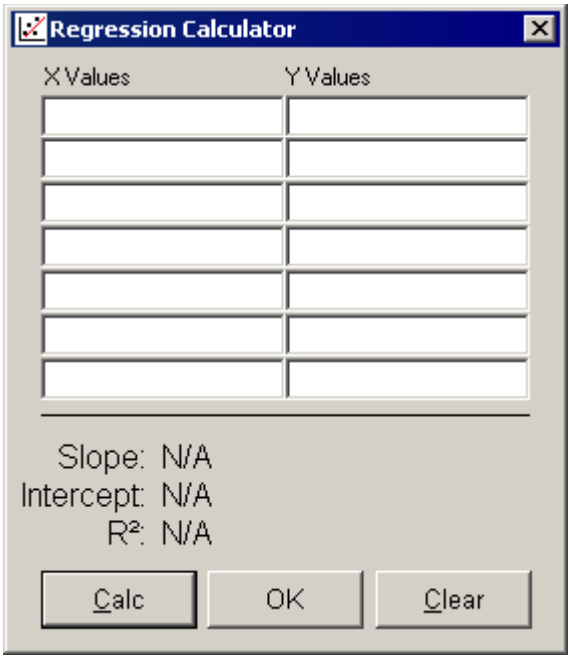


Figure 1-37 Regression Calculator dialog

2. Type data in pairs of x and y co-ordinates.
3. After entering two or more pairs, to get the Slope, Intercept, and R2 value, click **Calc**.
4. To delete the values for x and y co-ordinates, click **Clear**.
5. To exit the application, click **OK**.

Remove Graph Selections

Use this script to clear a selected area in the graph. For example, you can clear the graph selection line from the graph.

To use the script

- To remove selections from the graph, click **Script** > **RemoveGraphSelections**.

Repeat IDA Method

Use this script on an acquisition workstation. It opens the acquisition method for the data file and updates the exclusion list with the masses and times that have been acquired. The method is saved under the same name.

To use the script

1. In Explore mode, open an IDA data file.
2. Click **Script** > **RepeatIDAMethod**.
3. To keep the previous exclusion list, press the Ctrl key while clicking the script.

Savitzky-Golay Smooth

Using the Analyst® software, you can smooth a graph in Explore mode using two different algorithms. When you are using the IntelliQuan integration algorithm in Quantitate mode, a third smoothing algorithm, the Savitzky-Golay smooth, is available. The active graph in the Analyst software will be replaced by the smoothed graph.

The Savitzky-Golay Smooth script smooths the current active graph using the Savitzky-Golay smoothing algorithm. The IntelliQuan algorithm in Quantitate mode performs the smoothing process.

To use the script

1. With the peaks of interest selected in the active spectrum, click **Script** > **Savitzky-Golay Smooth**.

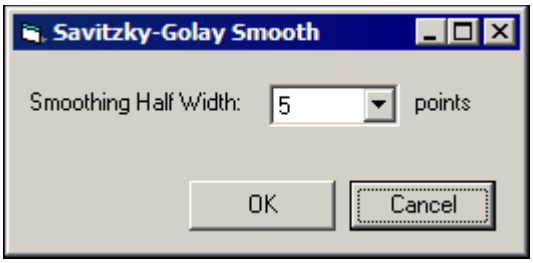


Figure 1-38 Savitzky-Golay Smooth dialog

2. Use the **Smoothing Half Width** drop-down list to set the half-width for smoothing the data. The total width will be twice this value plus one. This parameter is the same as the smoothing parameter used with the IntelliQuan algorithm in the Analyst software.
3. To perform the smoothing, click **OK**.

Selection Average and Standard Deviation

Use this script to calculate the average intensity and standard deviation of a selection in a graph for both spectral and chromatographic data. The graph is labeled with both the average and standard deviation of the selection.

To use the script

- Select either an active spectrum or active chromatogram, and click **Script** > **Selection Average and StdDev** script.

  The graph will be labeled with the average and standard deviation of the selection.

Known Issues and Limitations

This script will work only once for each graph. Use the following procedure if you want to run the script on the same graph more than once.

To calculate the average and standard deviation of a graph more than once

1. Copy the graph into a new pane. Click **Explore** > **Duplicate Data** > **Same Window**.
2. Make a selection in the new graph pane and run the **Selection Average and StdDev** script again.

Send to ACD SpecManager

This script is similar to the Export to JCamp script. However, instead of prompting for the name of the JCamp file, the spectra are exported to a temporary file and then sent directly to the ACD SpecManager application. There is no need to open this temporary file directly.

Prerequisites

You must have the SpecManager software installed.

To use the script

The first time you run the script, you are prompted to locate SpecManager. You will not be prompted again unless you press the Ctrl key while clicking the script.

Note: This script cannot be used with batch acquisition.

1. With either a chromatogram or a spectrum in an active pane, click **Script** > **Send to ACD SpecManager**.

   The following figure shows the options dialog that appears when you run either of the scripts to process chromatographic data. If you are interactively processing a single spectrum active in the Analyst® software, these options do not apply.

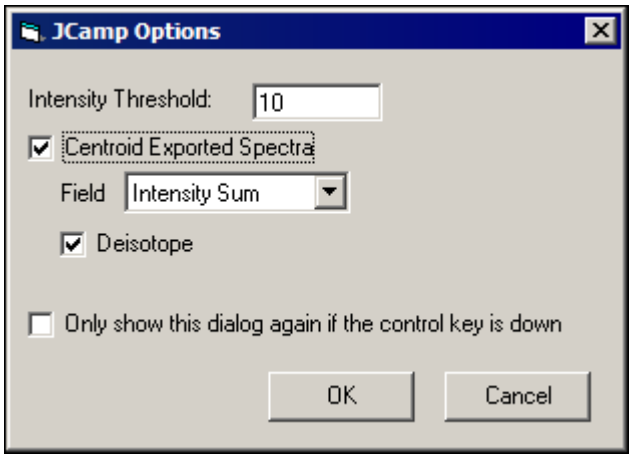


Figure 1-39 JCamp Options dialog

2. To centroid the exported spectra, select the **Centroid Exported Spectra** check box. This option reduces the size of the exported JCamp file.
3. To select the threshold that will be applied to the exported spectra, in the **Threshold** field, type a value. If you do not want to use a threshold, type 0 in the Intensity Threshold field.
4. Select the **Only show this dialog again if the control key is down** check box to have the JCAMP Options dialog appear if the Ctrl is pressed when selecting the script from the **Script** menu or when submitting the batch to the queue.

5. Click **OK** to continue processing and to have the spectra exported. These values are used as defaults until you change them again.
6. To close the dialog without making any changes, click **Cancel**. In the case of interactive use, canceling the dialog will also stop the export operation. However, in the case of batch operation, the batch will still be acquired and JCamp files will be exported using the original parameters.

Signal-to-Noise Using Peak-to-Peak

The Analyst® software calculates the signal-to-noise ratio by taking the standard deviation of all the chromatographic data points between the specified background start and background end times.

Use this processing script to calculate the signal-to-noise ratio for the active chromatogram. The script first subtracts the average background signal from the selected peak and then divides the subtracted signal by the peak-to-peak noise level. It then differentiates the noise and peak regions based on the maximum intensities of each region. Upon completion, the active chromatogram is labeled with the signal-to-noise ratio.

To use the script

- With a noise region and the peak of interest selected in the active chromatogram, click **Script** > **S-to-N**.

  The signal-to-noise ratio is calculated and the graph is labeled.

Tip! To remove the labels, press the Ctrl key while clicking the script.

Related Scripts

S_NstdDevQS: Calculates the signal-to-noise value with a method that uses the noise regions standard deviation.

Signal-to-Noise Using Standard Deviation

Use this script to calculate the signal-to-noise ratio of chromatographic peaks and label them. The script requires two regions to be selected on the chromatogram: a selection/region containing the noise region and a selection/region containing the peak of interest. The script will automatically determine which region contains the peak and the noise based on maximum intensities in each selection. It subtracts the average background signal intensity from the peak signal intensity and then divides the subtracted signal by a user-specified factor times the standard deviation of the noise region.

To use the script

1. With a noise region and the peak of interest selected in the active chromatogram, click **Script** > **S_NstdDevQS**.

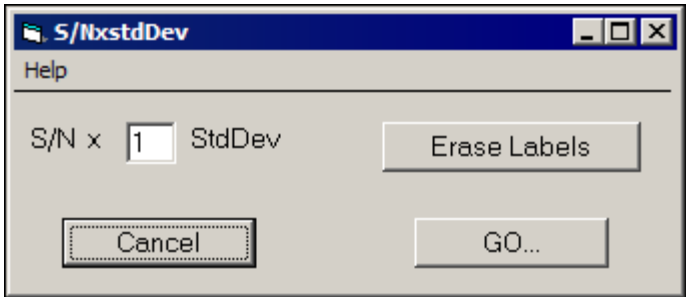


Figure 1-40 S/NxstdDev dialog

2. To erase any labels current on the active chromatogram, click **Erase Labels**.
3. To calculate the signal-to-noise ratio and label the graph, click **Go**.

Related Scripts

S-to-N using Peak-to-PeakS:Calculates the signal-to-noise for an active chromatogram. The background subtracted signal is divided by the peak-to-peak noise level.

Split Graph Script

Use this script to split a spectrum or chromatogram into a specified number of panes. Each resulting pane displays a proportional fraction of the total mass (or time) range. For example, if a spectrum displaying a mass range of 100 to 400 amu is split into three, the original spectrum will display a range of 100 to 200 amu, the second (new) spectrum will display 200 to 300 amu, and the third (new) spectrum 300 to 400 amu. The intention is to allow the maximum possible number of peaks to be labeled for subsequent printout.

To use the script

1. With either a chromatogram or a spectrum in an active pane, click **Script** > **SplitGraph**.

   Note: Make sure that the mass (or time) range for splitting is displayed. If necessary, dock the graph to make sure that the whole graph can be seen.

2. Do one of the following:
   - The current version of XICfromTable script can handle a maximum of 25 XIC mass ranges. If you press the Ctrl key while clicking the script, a dialog appears prompting you to select the number of panes to create. The preset value is four.
   - If the Ctrl key is not pressed, then the last number typed in the dialog is used.

Subtract Control Data from Sample Data

Use this script when the sample data of interest are in the active graph. The data may be any spectrum, TIC, or ADC trace. The script determines the data type, retrieves the corresponding data from the control file, and then displays the subtracted data in a graph.

You may select to overlay the subtracted data with sample data or control data.

To use the script

1. To process the data with current preferences, click **Script** > **SubtractControlData**.
2. Do one of the following:
   - To see the script description, set the processing preferences, and get the subtracted data, hold down the Shift key when clicking the script.
   - To update the processing preferences and get the subtracted data, press the Ctrl key when clicking the script.

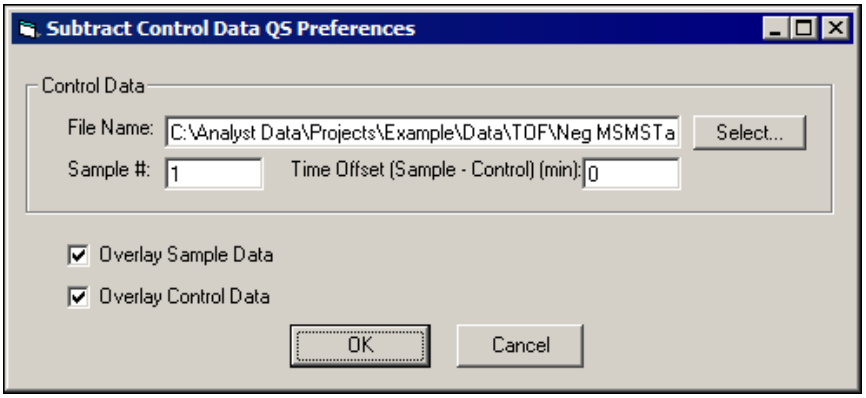


Figure 1-41 Subtract Control Data QS Preferences dialog

Unit Conversion

Use this processing script to convert from one set of concentration units to another.

To use the script

1. Click **Script** > **Unit Conversion**.

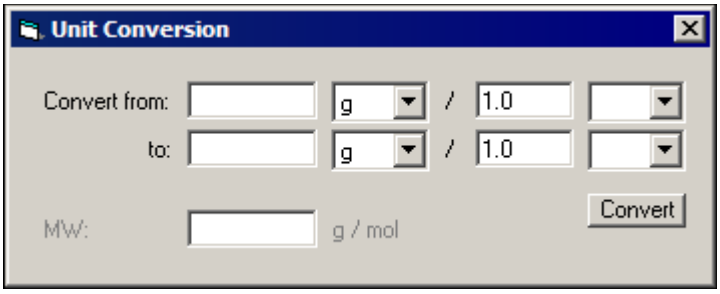


Figure 1-42 Unit Conversion dialog

2. In the **Convert from** field, type the concentration and units to be converted.
3. If the conversion is from a weight-based concentration (for example, g/L) to a molar-based concentration (for example, mol/L), then in the MW field, type the molecular weight of the component.
4. In the **Convert to** field, type the unit.
5. To perform the conversion, click **Convert**.

   The calculated values will be displayed in the **Convert to** field.
6. To retrieve these values, press Ctrl +C to select and copy them to the clipboard. These values can then be pasted into another application.

Wiff To MatLab

Use this script to extract the data from a .wiff file and creates the following matrices in a MatLab file: Data, Masses, Wavelengths, Scans, and Filename. For more information about these matrices, see Table 1-8 *Matrix Definitions* on page 82. This MatLab file can then be included in a MatLab script and used to compute and display the results.

Although you can use the Analyst® software to perform various data manipulations, you may use this specialized script, Wiff to MatLab, for data computations. The Analyst software cannot extract data from a .wiff file and then store it in a file that can be read and interpreted by MatLab.

In addition to exporting the mass spectral data, this script can also export data from a diode array detector (DAD).

The script will create a single MatLab .mat file for each sample to be translated. Depending on the type of mass spectra data and the user options, the script can create data in two different formats:

- The first format saves the intensities in a matrix of size (number of masses) x (number of scans). There is thus an entry in the matrix for every mass that was scanned and every spectrum. This is the default format for quadrupole spectra and the format that is always used for diode array data.
- The second format is sparse or compressed and allows data points that have an intensity of zero to be omitted; depending on the number of such data points in the original .wiff sample, this can potentially greatly reduce the size of the MatLab file. This format uses a matrix of size 2 x (total number of (mass, intensity) pairs). The first row represents masses and the second row the intensities. A given number of initial columns corresponds to the first spectrum, a given number of following ones to the second spectrum, and so forth. Each (sparse) spectrum is essentially stored end-to-end.

A separate vector is written, which you can use to determine the starting and ending position of any given spectrum in this matrix. This vector contains the one-based index of the start of a given spectrum. The end for a given spectrum can be determined by subtracting one from the start of the following spectrum (except for the very last spectrum, which is determined by the size of the above-mentioned matrix).

The first format is the default format for quadrupole spectra and is always used for diode array data. The second (sparse) format is always used for TOF (time-of-flight) data and can optionally be used for quadrupole data.

The actual names of the various matrices are specified using the Options dialog described later in this document.

To use the script

1. With a chromatogram in an active pane, click **Script** > **Wiff to MatLab**. Alternatively, this script can be attached to a batch; select this script using the Batch Editor.

   If the chromatogram is associated with mass spectral data (TIC, XIC) then MS data is exported to the MatLab file; if the chromatogram is associated with diode array data (TWC, XWC) then DAD data is exported.

2. To change the conversion options, open the **Wiff to MatLab Conversion Options** dialog by pressing the Ctrl key while clicking the script. Otherwise, the options previously specified are used.

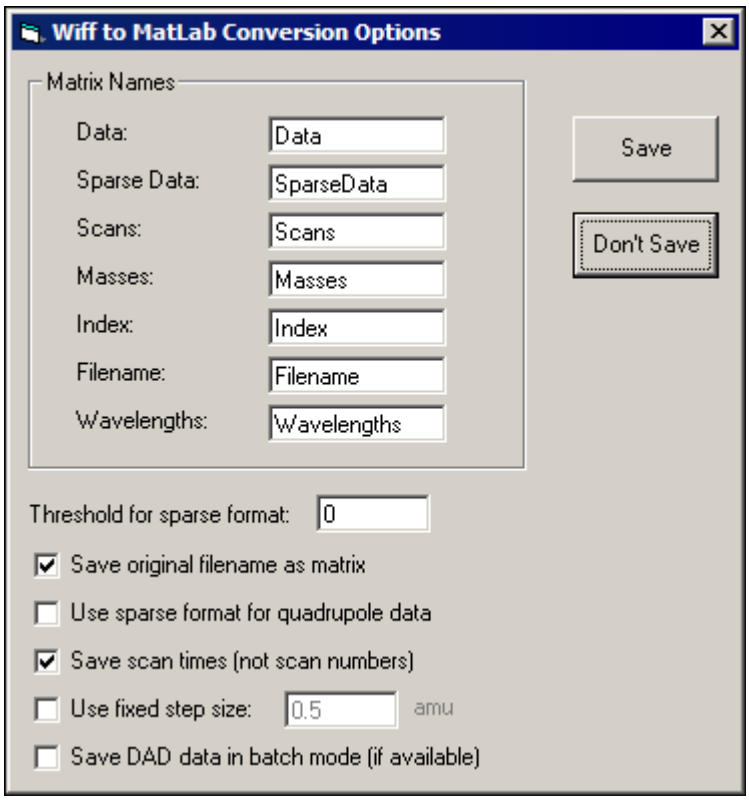


Figure 1-43 Wiff to MatLab Conversion Options dialog

3. In the **Matrix Names** fields, type the names of the matrices that the script produces. It is recommended that you keep these names as their default values.
4. In the **Threshold for sparse format** field, type a value that will be used to reduce the size of the output MatLab file. For the sparse format only, only (mass, intensity) pairs with intensity larger than the specified value will be written.
5. If the **Save original filename as matrix** field is selected, then the script will create and populate the Filename matrix.
6. To have the quadrupole spectra saved in the sparse format, select the **Use sparse format for quadrupole data** check box. Note that TOF spectra are always saved in the sparse format and that diode array data is always saved in the non-sparse format.
7. If the **Save scan times (not scan numbers)** field is cleared, then the script will populate the Scans matrix with the time in seconds for each scan. Otherwise, the Scans matrix is populated with the scan numbers.
8. In the **Use fixed step size** field, type the step size of the data to extract. If this field is cleared, then the acquisition step size is used.
9. To populate the wavelengths matrix with the DAD data when the script is attached to a batch, select **Save DAD data in batch mode (if available)**.
10. To save these settings and continue processing the data, click **Save**.
11. To discard any changes made to the settings, click **Don't Save**. Data processing will continue after you click this button.

12. To automatically generate a MatLab file for every sample submitted as part of a batch run in the Batch Editor, click **Select Script** to select the script before submitting the batch to the queue. The script should be located in the Processing Scripts subproject of the API Instrument project.

    A MatLab file is created for every different experiment for each sample in the batch. The MatLab files are placed in the same location as the data files and will have the same names with the .wiff extension replaced by .mat; however, the index of the sample within the .wiff file will be appended. In addition, for samples acquired using acquisition methods containing more than one experiment, the period and experiment number will be appended to the MatLab filename.

    For example if a data file is called test.wiff and contains two samples, the MatLab files will be called:

    - test-1.mat
    - test-2.mat

    If the acquisition method contained one period and two experiments, four MatLab files would be generated (two for each sample):

    - test-1(1,1).mat
    - test-1(1,2).mat
    - test-2(1,1).mat
    - test-2(1,2).mat.

    If the data file contains diode array data and the Save DAD data check box is selected, then an additional file with the sample index and (DAD) is created. For the previous example, files called test-1(DAD) and test-2(DAD).mat are created.

Known Issues and Limitations

When attaching this script to a batch, make sure that the Acquisition Queue window is opened before submitting the batch. This window must be open until the acquisition has completed to make sure that the script is working properly.

Related Scripts

Export to JCamp: Converts spectra from .wiff format to JCamp format.

Table 1-8 Matrix Definitions

Matrix	Dimensions	Type	Descriptions
Data	Number of masses (or wavelengths) x number of scans	Float	The raw intensities for all of the spectra.
SparseData	2 x total number of (mass, intensity) pairs	Double	The raw masses (first row) and intensities (second row) for all spectra. This matrix is used only with the sparse format.
Masses	Number of masses x 1	Float	The actual *m/z* values scanned by the instrument. This matrix is only present when exporting MS data.

Table 1-8 Matrix Definitions (Continued)

Matrix	Dimensions	Type	Descriptions
Wavelengths	Number of wavelengths x 1	Float	The actual wavelengths acquired by the diode array detector. This matrix is present only when exporting DAD data.
Index	1 x number of scans	Long	
Scans	1 x number of scans	Float	The retention times (in seconds) or the scan numbers for the spectra.
Filename	1 x length of filename	Text	This optional matrix specifies the filename of the original data file.

XIC from BPC

Use this script to retrieve the list of base peak masses and overlay the corresponding extracted ion chromatograms in an Explore pane below the active pane. Run the XIC From Base Peak Masses script after selecting a time region of interest in a chromatogram. The largest peak in each XIC trace will be labeled with its mass if it is greater than 5% of the most intense XIC peak.

To use the script

You may optionally select to overlay the XIC traces with an ADC trace. The ADC trace will be normalized to the most intense XIC trace.

1. To process the data with current preferences, click **Script** > **XIC from BPC**
2. Do one of the following:
   - To see the script description, set the processing preferences, and get the overlaid XIC traces, hold the Shift key down while clicking the script.
   - To update the processing preferences and get the overlaid XIC traces, press the Ctrl key while clicking the script.

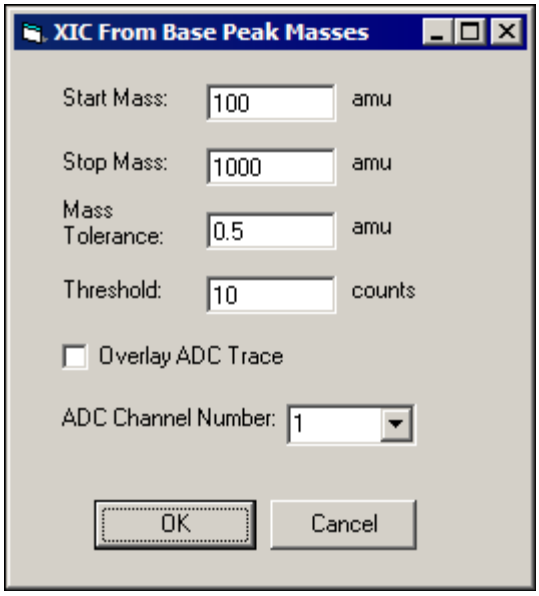


Figure 1-44 XIC From Base Peak Masses dialog

When processing is started, a progress bar indicates the current step. When finished, a new pane with overlaid XIC traces is the active pane in the Analyst® software document. If you want to use the Cycle Overlays feature and it is unavailable, switch the active pane in the Analyst software to another one and then reselect the overlaid XIC pane.

XIC from Table

Use this script to create and read start and stop masses from a file. The file will have two columns separated by a tab. The first column contains the start mass and second column contains the stop mass.

When working with full scan data, the Analyst® software can show an Extracted Ion Chromatogram (XIC) for a subset of the scanned mass range. If the subsets are repeatedly used on different data, it is often convenient to store the subsets of the mass range, consisting of a start and stop mass, in an external file and have the Analyst software generate an XIC based on this file.

The script generates the requested XICs either as one XIC per pane or all the XICs overlaid in one pane from the current Total Ion Chromatogram (TIC).

To use the script

1. With a TIC open in an active pane, click **Script > XIC_from_table**.

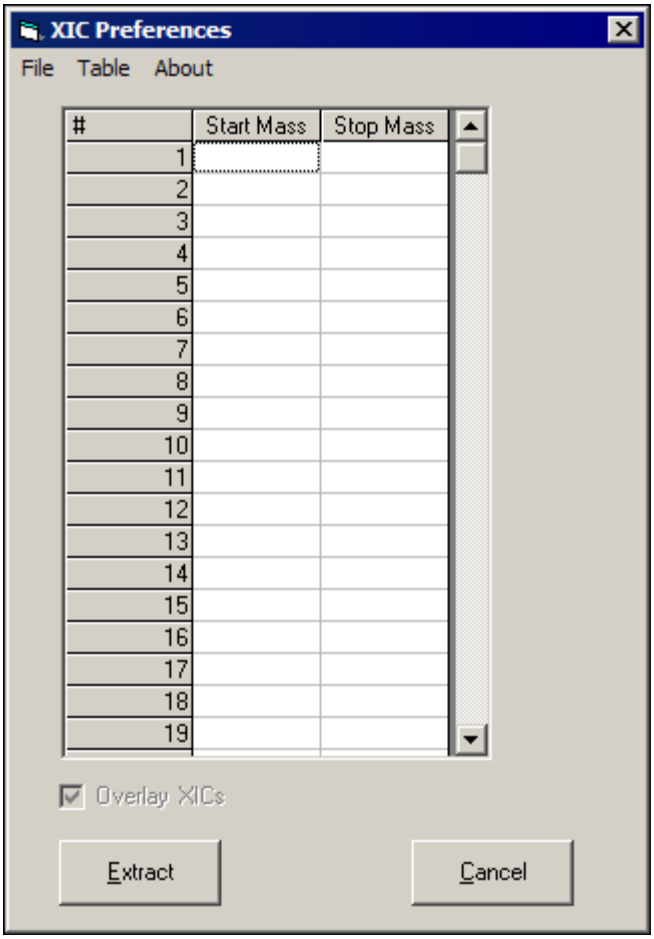


Figure 1-45 XIC Preferences dialog

2. Using the grid in the dialog, type the mass ranges to be extracted into an XIC. To populate the grid from a text file, click **File** > **Open** and then navigate to the text file.
3. To save the current information in the grid to a file, click **File** > **Save As**.

4. To clear the grid of all entries, click **Table** > **Clear**.
5. To overlay all XICs, into a single pane, select **Overlay XICs** check box.
6. To create all the specified XICs, click **Extract**.
7. To close the dialog and close the script, click **Cancel**.

Known Issues and Limitations

- When not loading XIC start/stop masses from a file, the maximum number of mass pairs you can type is 25. However, when loading from a text file, an unlimited number of start/stop mass pairs can be specified.
- Due to limited space on the screen, you may have to select the **Overlay XICs** check box when creating more than six XICs.